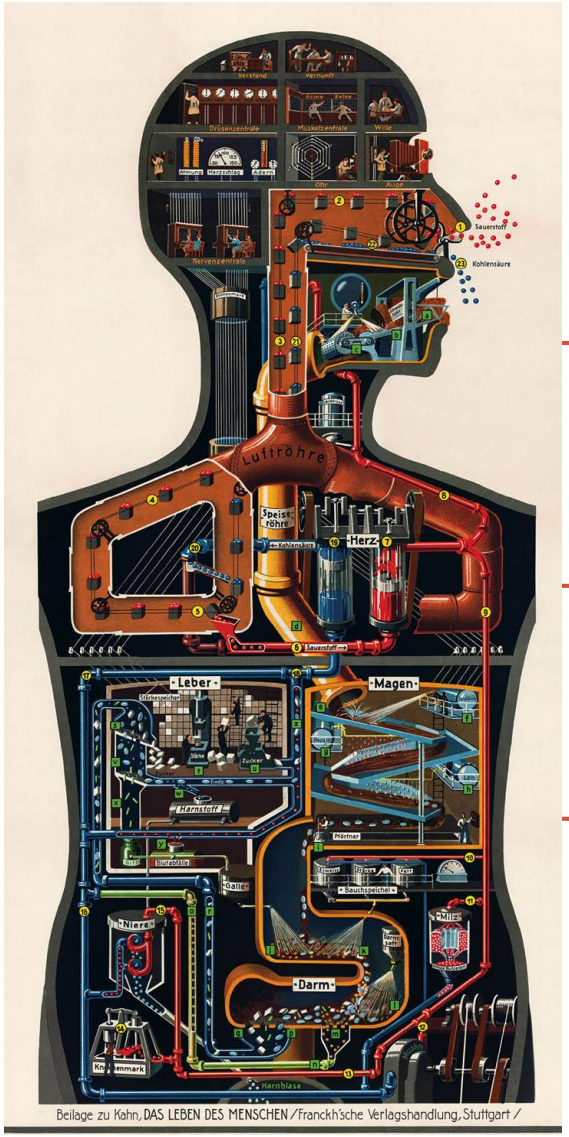


Fritz Kahn (1888 – 1968)

BIOMECHANICS

5: Tissue Engineering

7ο εξάμηνο

Σχολή Μηχανολόγων Μηχανικών ΕΜΠ

Διδάσκων:

Michael Neidlin

What is Tissue Engineering?

The field which applies principles of **Biology** and **Engineering** to the development of **functional substitutes** for **damaged tissues**(Langer et al. 1993).

More info: **https://www.youtube.com/watch?v=7Q3S6q97FiU**

The 4 components of Tissue Engineering

BIOREACTORS

Stress/strain controlled, Shear Flow, Microgravity, Perfusion, Regular incubator etc

CELLS

Autologous, Allogeneic,

Differentiated , Stem Cells

SCAFFOLDS

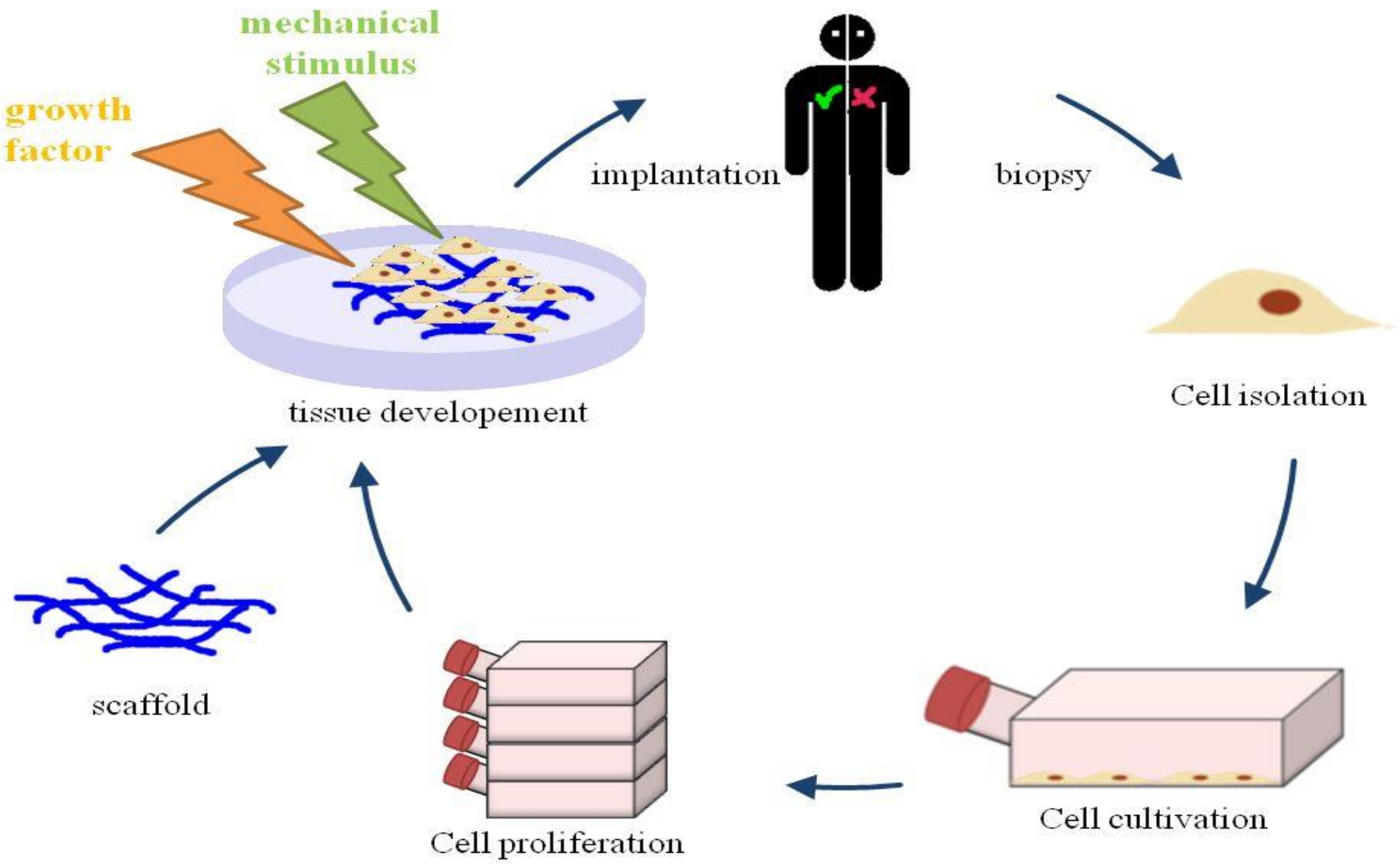
Synthetic (PLA, PGA, etc)

Natural polymers (Alginate, Collagen, HA, etc)

BIOACTIVE ENVIRONMENT

Growth factors , Hormones Cytokines , Mechanical environment

Process of Tissue Engineering



https://en.wikipedia.org/wiki/Tissue_engineering

Process of Tissue Engineering

1. Initially, **cells** are being isolated from the patient.
2. Secondly, those cells are cultivated until a “cell bank” is created.
3. Then, different biomaterials are utilized in order to fabricate **scaffolds.**
4. The next step is to seed the scaffold with cells.
5. The seeded scaffolds can also be bathed with growth factors, as well as they can be mechanically altered (compressive load, hydrostatic pressure) in order to stimulate cellular growth and proliferation **(bioactive environment).**
6. Finally, the engineered scaffolds along with the cells are placed in a **bioreactor,** a device which mimics the conditions inside the body (Lanza et al. 2007).

Video showing bioreacors for heart valve scaffold & tendon
https://www.youtube.com/watch?v=EFm9qbhsL_A

https://en.wikipedia.org/wiki/Tissue_engineering

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1. Stem Cells

What are Stem Cells?

Stem cells are cells that

(1) can **self-renew** (replicate while maintaining the undifferentiated state)

(2) have the **potential to differentiate**

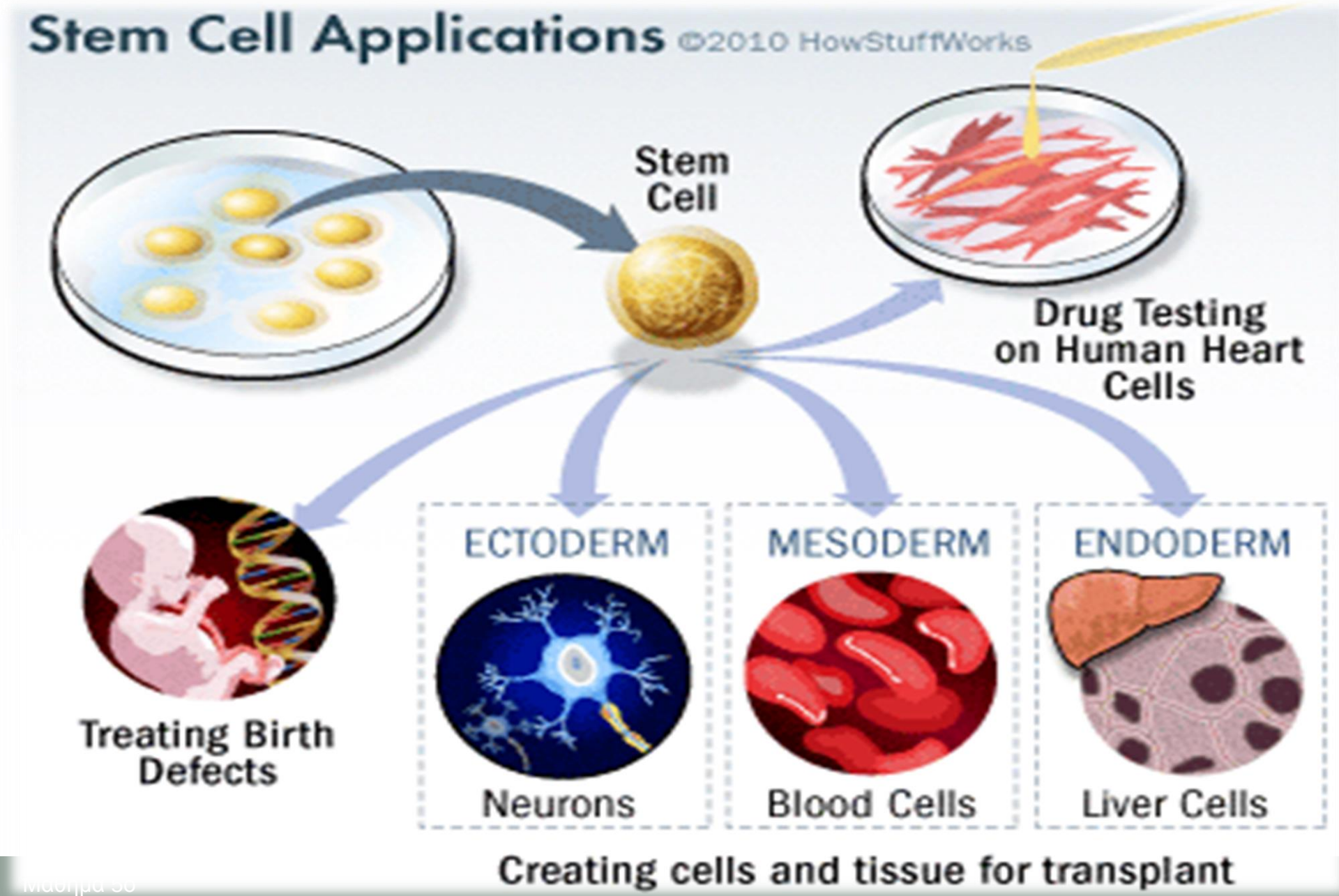
They can be **embryonic** or **adult**

Potency specifies the differentiation potential (Bernhard Palsson, 2004):

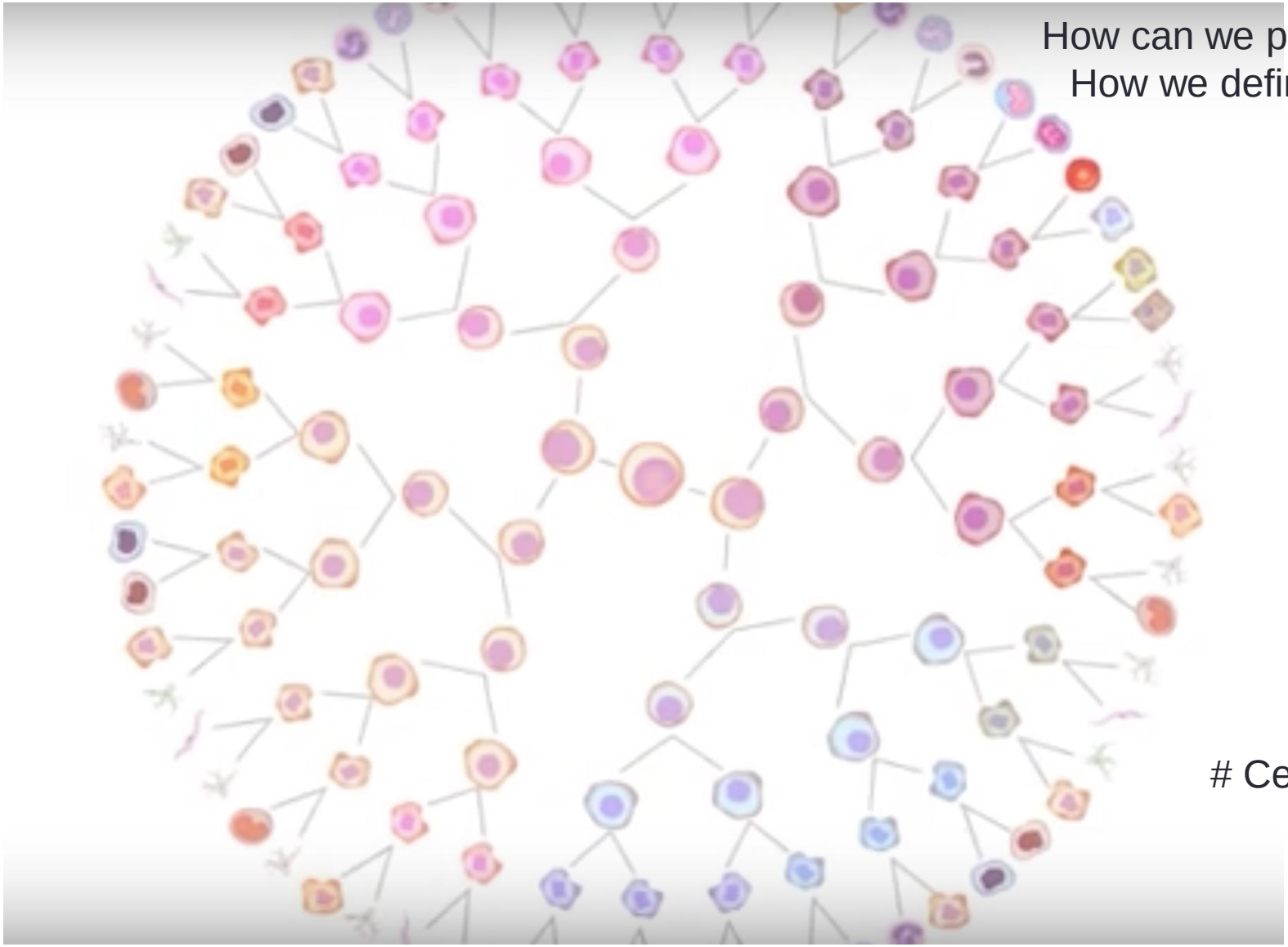
1. ***Totipotent*** → *Can produce all cell types (i.e. embryonic SC)*
2. ***Pluripotent*** → *Can produce most cell types (i.e. umbilical cord SC)*
3. ***Unipotent*** → *Can produce one cell type (i.e. adult SC)*

An illustrating video explaining what stem cells are:
https://www.youtube.com/watch?v=evH0I7Coc54

1. Stem Cells


Stem Cell Applications ©2010 HowStuffWorks
Stem Cell
Drug Testing on Human Heart Cells
Treating Birth Defects
ECTODERM
MESODERM
ENDODERM
Neurons
Blood Cells
Liver Cells
Creating cells and tissue for transplant

1. Stem Cells (lineages)



How can we program them?
How we define their state?

Cells: ~30 trillion
200 cell types

1. Stem Cells

What do stem cells look like?

They are **small spherical cells** that are **about 6 to 8 μm in diameter** and have **no particular morphological features**

Which tissues have stem cells?

Rapidly proliferating tissues (bone marrow, muscle and skin) as well as organs with slow turnover times (liver, brain, pancreas). Adipose tissue have also stems cells.

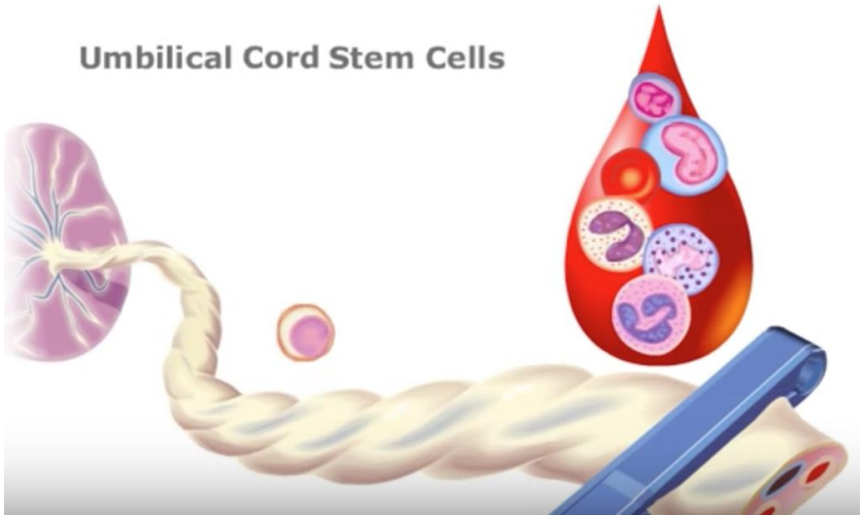
Stem cells build tissue

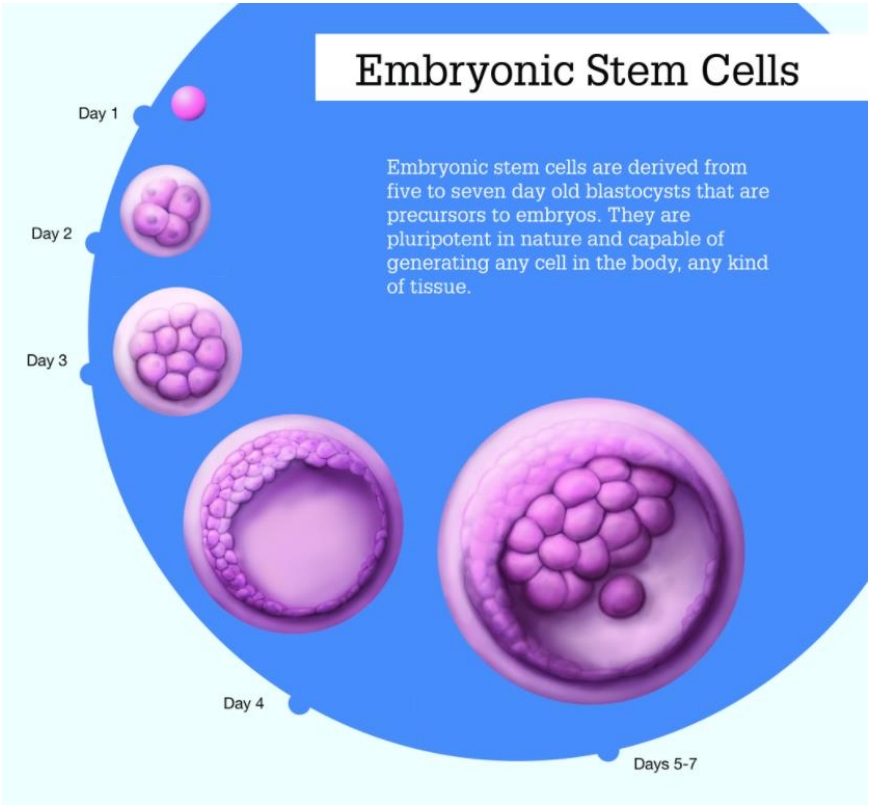
Stem-cell commitment along differentiated lineages underlies **organ function, repair and formation**. The cellular-fate processes of **cell proliferation, cell death, cell motion and cell differentiation** together determine the **fate of the committed cell.** Stem cells are the **starting material** for many key processes in tissue engineering and must be considered as an important source of regenerative potential (Bernhard Palsson, 2004).

1.1 Embryonic Stem Cells

- Embryonic stem cells (ESC) are derived from fertilized eggs formed during infertility treatment of couples for *in vitro* fertilization (Principles of Tissue Engineering, 2007).
- The **embryonic stem cells can divide indefinitely** (self-renew), apparently without limit and are pluripotent- that is, they can give rise to any cell type in the body (Amit et al., 2000; Evans and Kaufmann, 1981; G.R Martin, 1981).

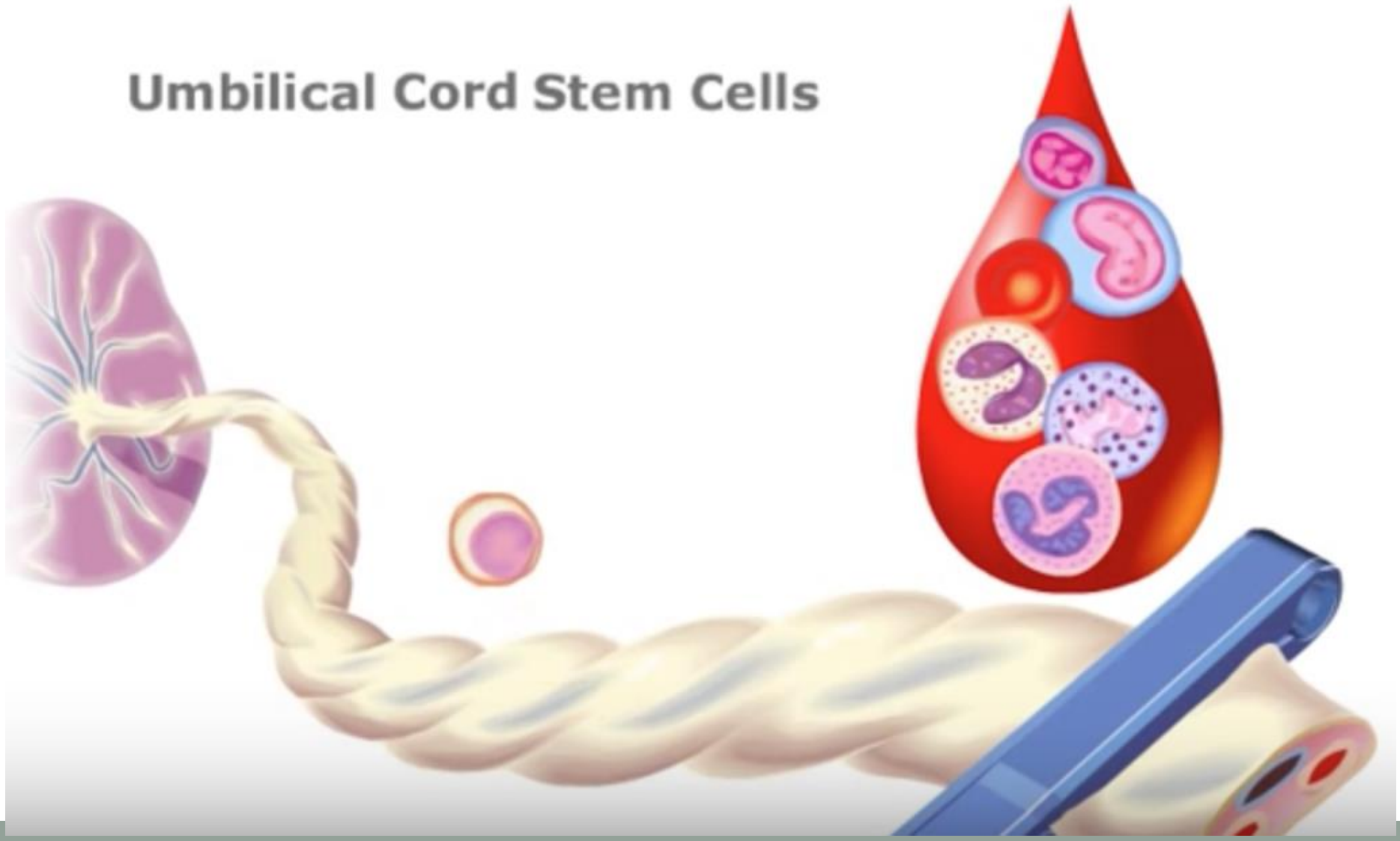
Illustrating video explaining what an ESC is:
https://www.youtube.com/watch?v=uC0fLXdu56g





1.1 Umbilical Cord Stem Cells

- Similar to Embryonic stem cells
- They divide indefinitely. They are pluripotent



Issues for using ESC

- **Histocompatibility:** ES cells from different donor can cause immune rejection. ES cells from the same donor is not possible!

- **GMP:** Clinical application for tissue engineering requires robust processes to isolate and grow them under GMP (good manufacturing practice) and regulatory review for safety.

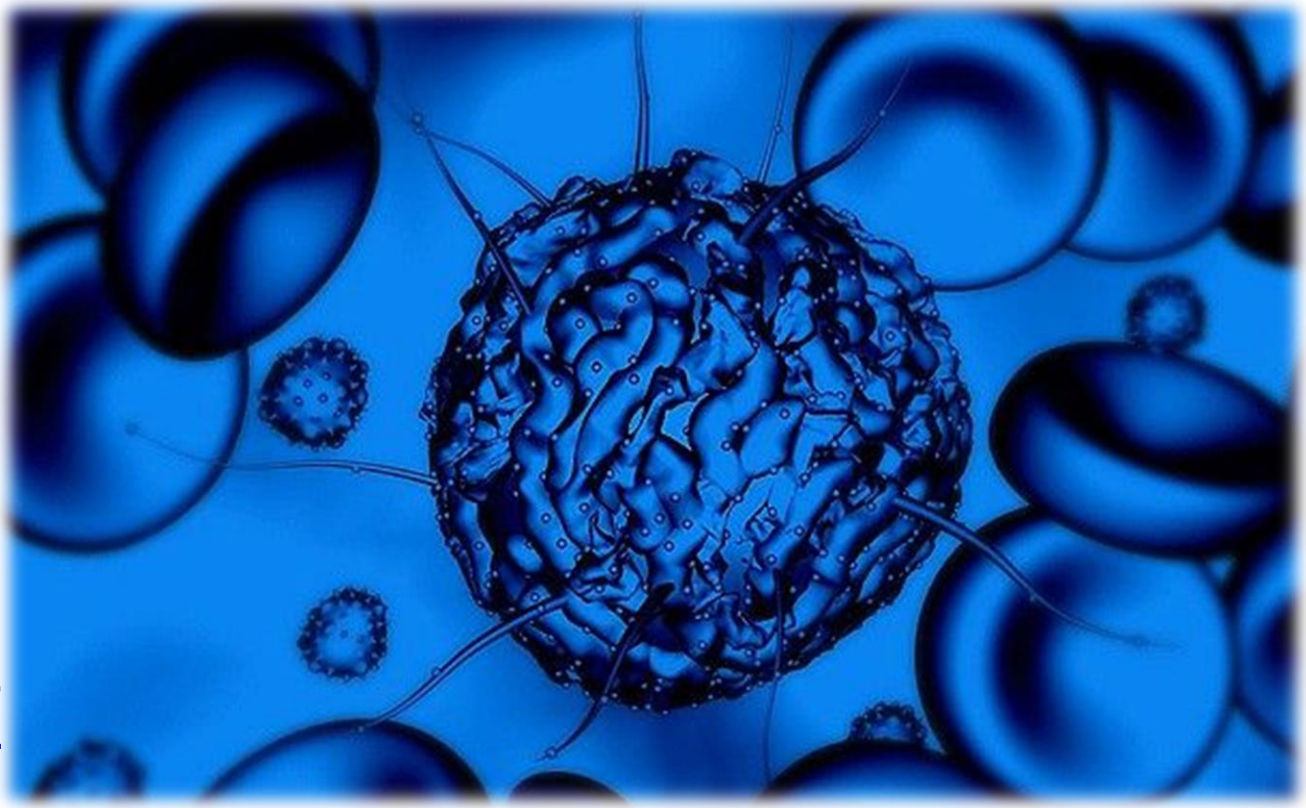
- The greater challenge remains in **directing the differentiation** of human ES cells to a given desired lineage with high efficiency.

1.2 Adult Stem Cells

- Adult stem cells are undifferentiated cell, found among differentiated cells in a tissue or organ. It can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ.

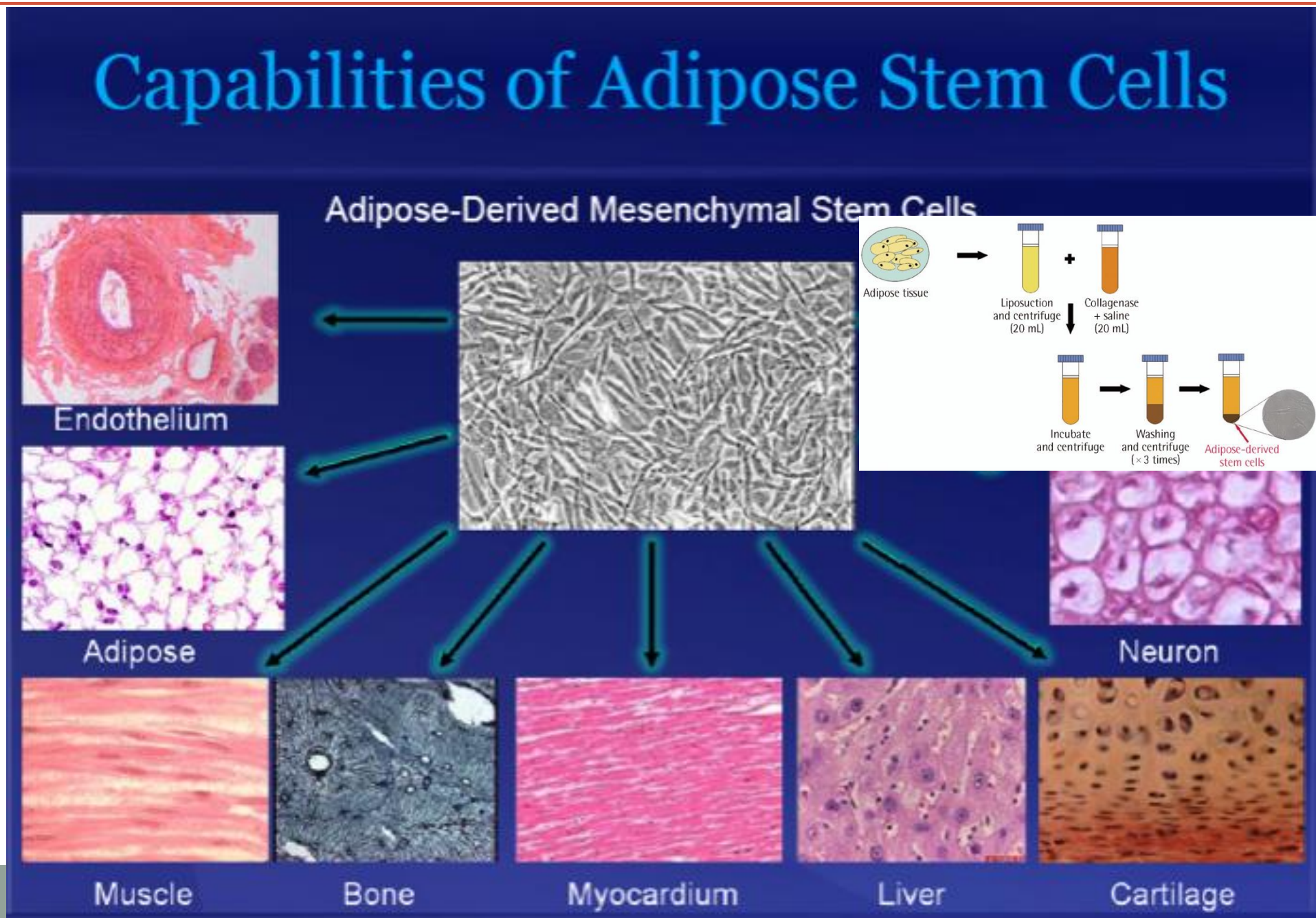
- The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found.

Illustrating video explaining what an adult stem cell is:
https://www.youtube.com/watch?v=kgHAUmV45Gc



Adult Neural Stem Cells. **Source: Cell Life Medical**

1.2 Example: Adipose derived stem cells



1.4 Embryonic vs Adult Stem Cells

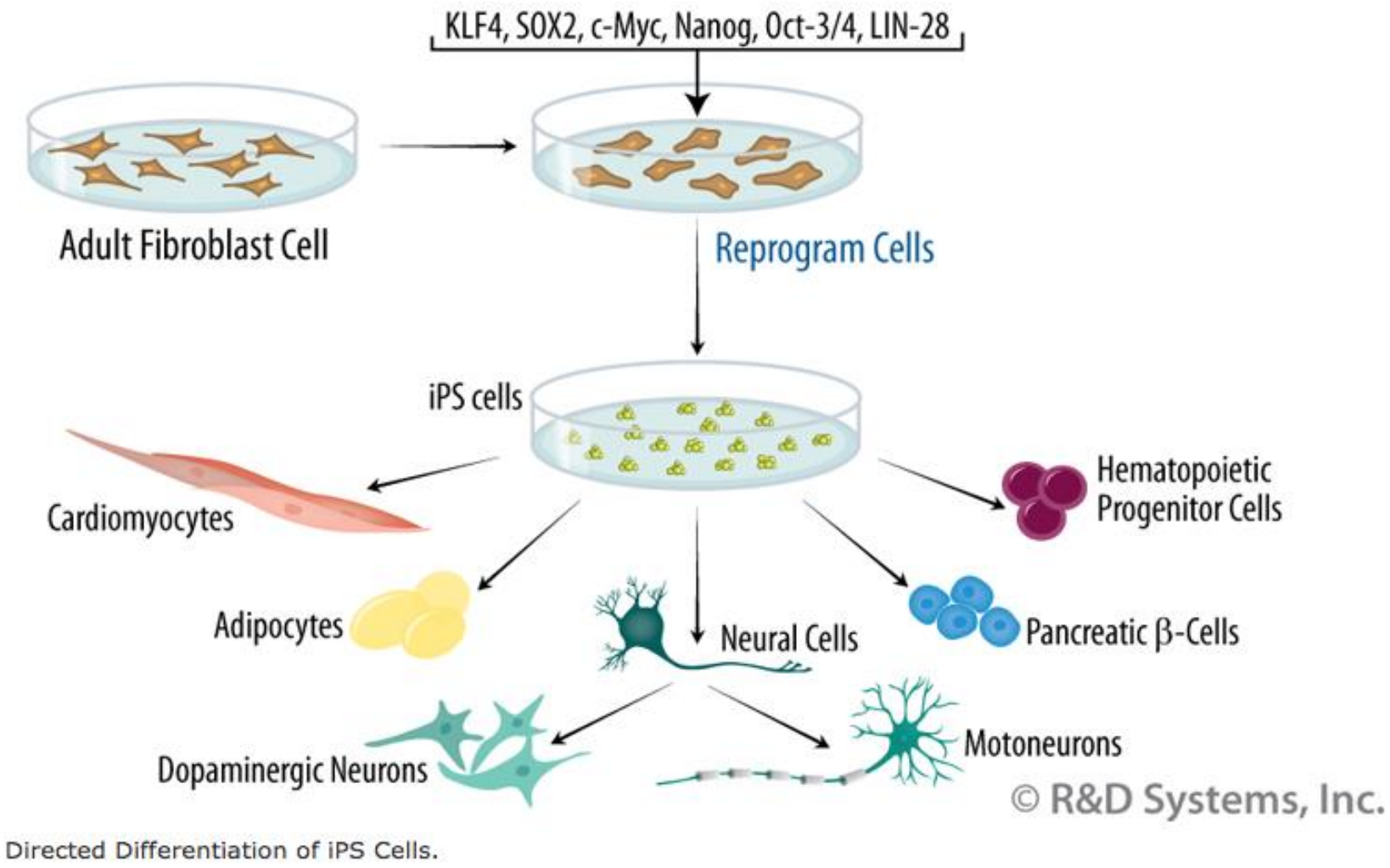
- Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin (Lanza et al., 2007).

- Embryonic stem cells can be grown relatively easily in culture.

- Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, and methods to expand their numbers in cell culture have not yet been worked out.

1.3 Induced Pluripotent Stem Cells

Genetically reprogrammed cells to an embryonic stem cell–like



Directed Differentiation of iPS Cells.

1.3 Induced Pluripotent Stem Cells

- Induced Pluripotent Stem Cells are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells (Lanza et al., 2007).

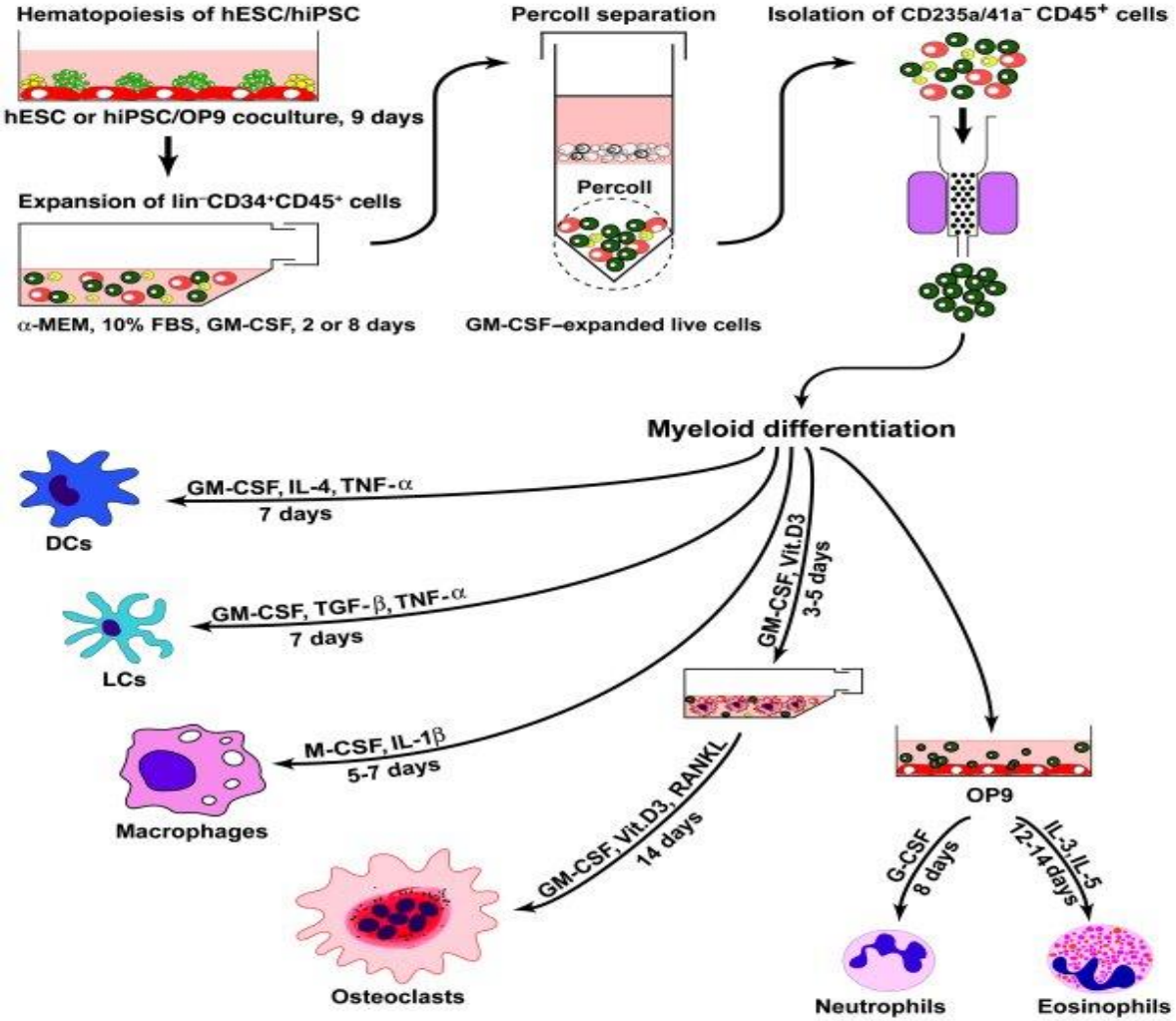
- Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007 (Lanza et al., 2007).

- Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases. The iPSC strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body (Lanza et al., 2007).

→ **Great importance of iPSC in drug discovery**

1.5 Cell Differentiation

- **Cellular differentiation:** is the process where a cell changes from one cell type to another. Most commonly this is a less specialized type becoming a more specialized type, such as during cell growth. Differentiation occurs numerous times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types.



Source: *Choi et al. ,2009*

1.6 Cell Sources

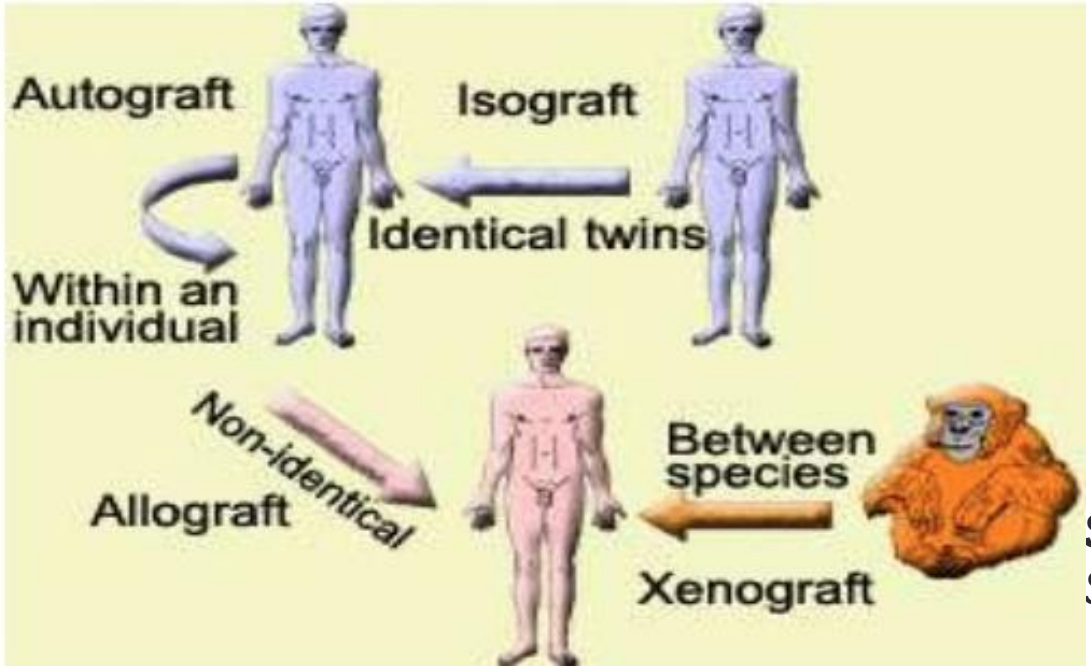
- **Autologous:** Come from the same person that needs the new cells.
- **Allogeneic:** Come from the same species but different donor
- **Xenogenic:** Come from a different species

Autologous graft (Autograft):

Syngeneic graft (Isograft):

Allogeneic graft (Homograft):

Xenogeneic graft (Heterograft):



Source: *SlideShare*

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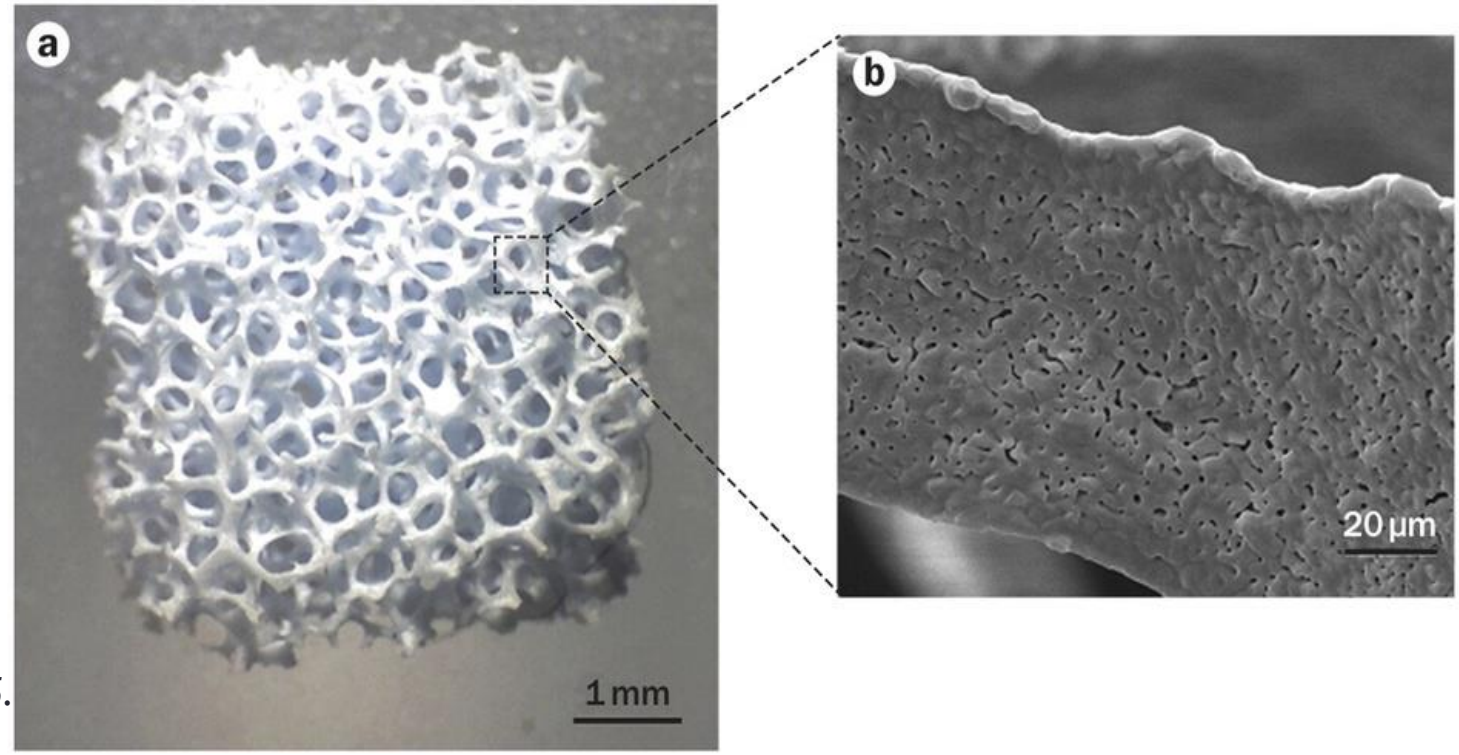
2. Scaffolds

- Scaffolds are **3-dimensional materials** constructed in order to provide **structure** to a developing tissue and to **allow cells** to adhere, proliferate, differentiate and most importantly, **secrete extracellular matrix (ECM)** (Leong MF. et al., 2009).
- Many different materials have been investigated in order to construct scaffolds such as polymers (PLA, PGA, PCL, PEG), bioactive ceramics (HA, TCP) as well as natural polymers (Collagen, GAGs, Chitosan).



Source:*Smith, B. D, 2015. Example of a fixed-shape bone scaffold.*

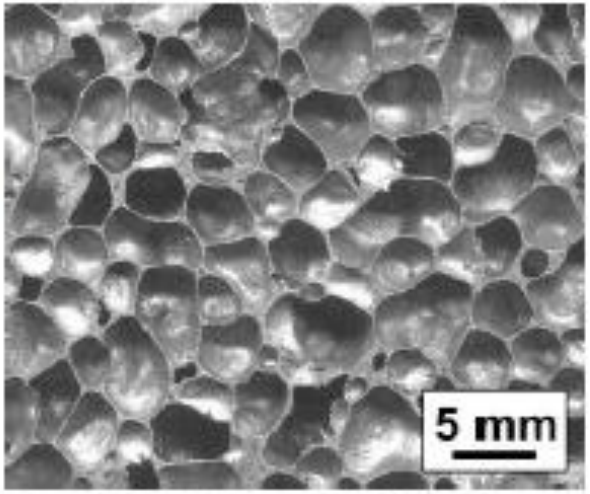
Nature Reviews | Rheumatology

2.1 Scaffold construction requirements

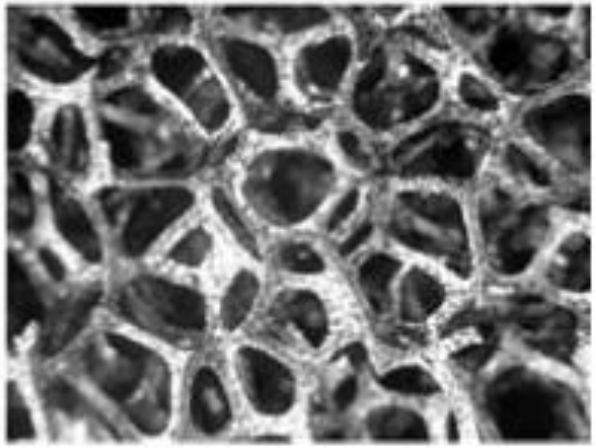
In order to engineer a successful scaffold, a plethora of requirements should be met, such as:

1. ***Architecture:*** **Pore Size diameter & connectivity (porous material)**

Closed foam plastic
(foardpanel.com)



Open foam plastic
(ultramet.com)



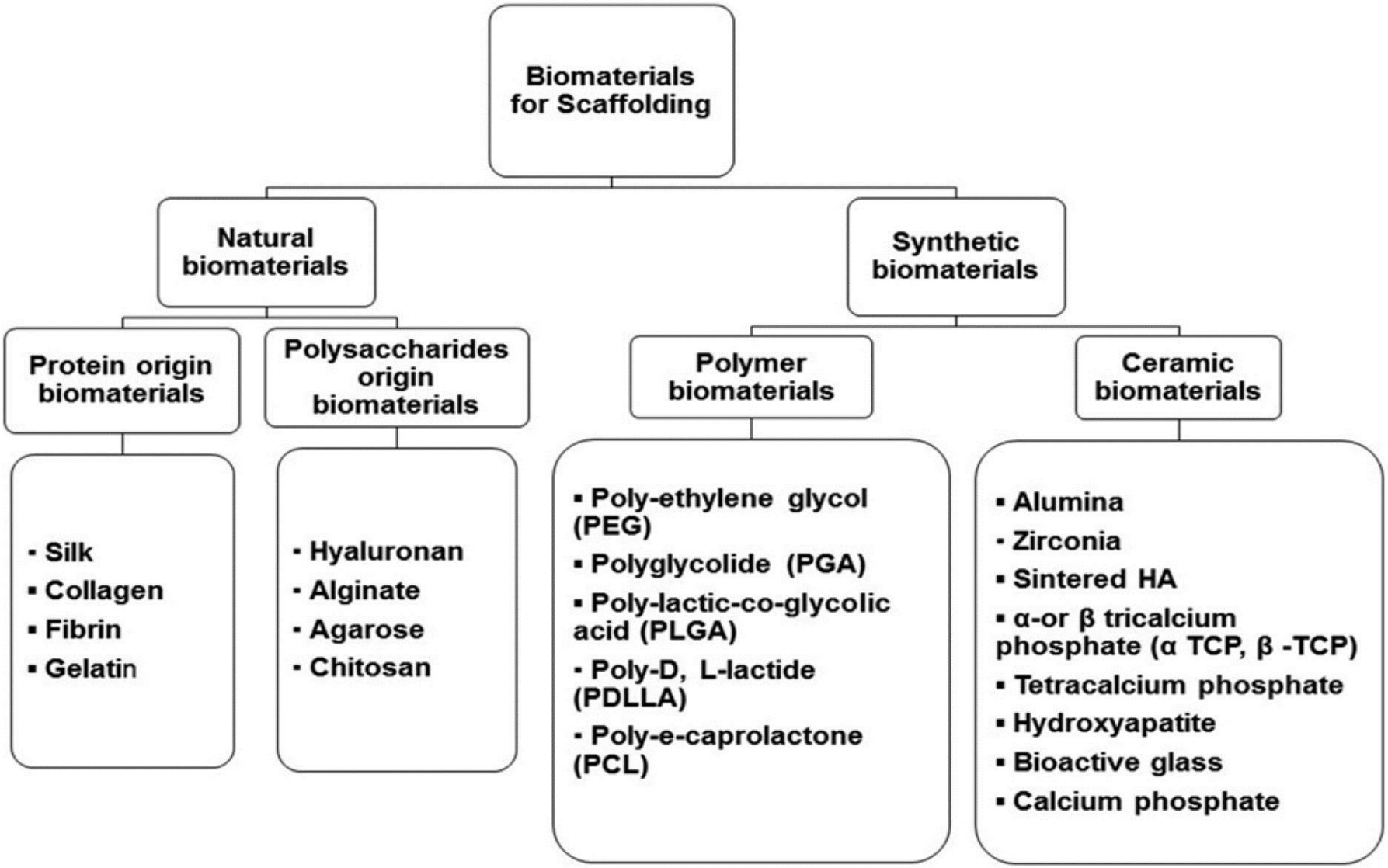
2.1 Scaffold construction requirements

2. ***Biocompatibility***

3. ***Bioactivity:*** Scaffolds may interact with the cellular components of the engineered tissues actively to facilitate and regulate their activities. The biomaterials may include biological cues such as cell-adhesive ligands to enhance attachment or physical cues such as topography to influence cell morphology and alignment. The scaffold may also serve as a delivery vehicle or reservoir for exogenous growth-stimulating signals such as growth factors to speed up regeneration (Discher DE, et al., 2005).

4. ***Mechanical property:*** Scaffolds provide mechanical and shape stability to the tissue defect. The intrinsic mechanical properties of the biomaterials used for scaffolding or their post-processing properties should match that of the host tissue. Exerting traction forces on a substrate, many mature cell types, such as epithelial cells, fibroblasts, muscle cells, and neurons, sense the stiffness of the substrate and show dissimilar morphology and adhesive characteristics (Engler AJ, et al., 2006).

2.2 Scaffolds synthesis



Source: *Alaribe et al, 2016*

2.3 Scaffolds construction

A number of different methods has been introduced for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none are free of drawbacks.

1. ***Nanofiber self-assembly:*** Molecular self-assembly is one of the few methods for creating biomaterials with properties similar in scale and chemistry to that of the natural *in vivo* extracellular matrix (ECM), a crucial step toward tissue engineering of complex tissues. Moreover, these hydrogel scaffolds have shown superiority in in vivo toxicology and biocompatibility compared to traditional macro scaffolds and animal-derived materials (Cassidy JW, 2014).
2. ***Textile technologies:*** These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular, non-woven polyglycolide structures have been tested for tissue engineering applications: such fibrous structures have been found useful to grow different types of cells. The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size(Ekevall E, 2004).

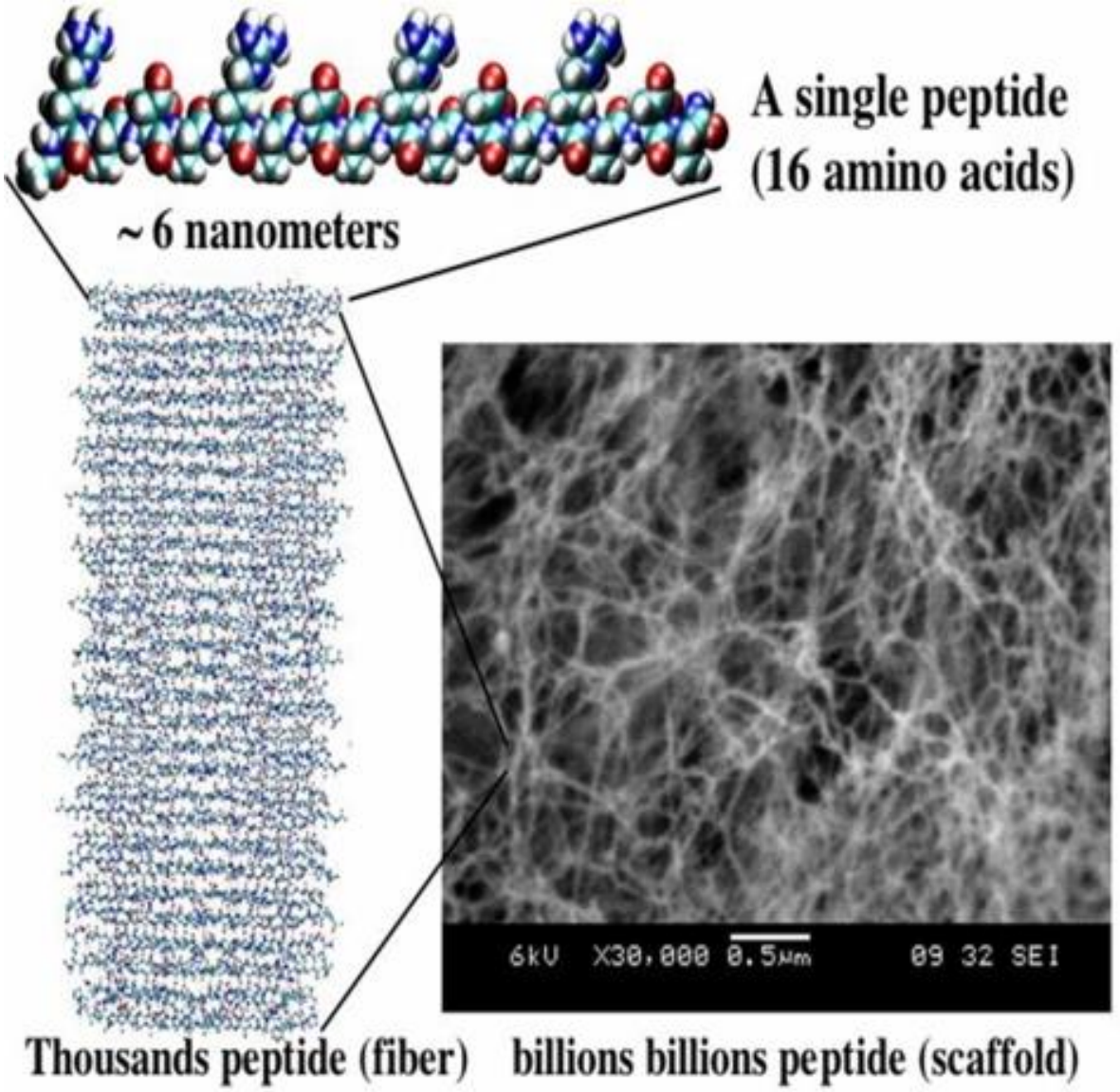
2.3 Scaffolds construction

3. ***Freeze- drying***: First, a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane) then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure (Haugh MG, 2010).

4. ***Electrospinning:*** In a typical electrospinning set-up, a solution is fed through a spinneret and a high voltage is applied to the tip. The buildup of electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream. At a laboratory level, a typical electrospinning set-up only requires a high voltage power supply (up to 30 kV), a syringe, a flat tip needle and a conducting collector. For these reasons, electrospinning has become a common method of scaffold manufacture in many labs. By modifying variables such as the distance to collector, magnitude of applied voltage, or solution flow rate researchers can dramatically change the overall scaffold architecture (Lannutti J, et al., 2007).

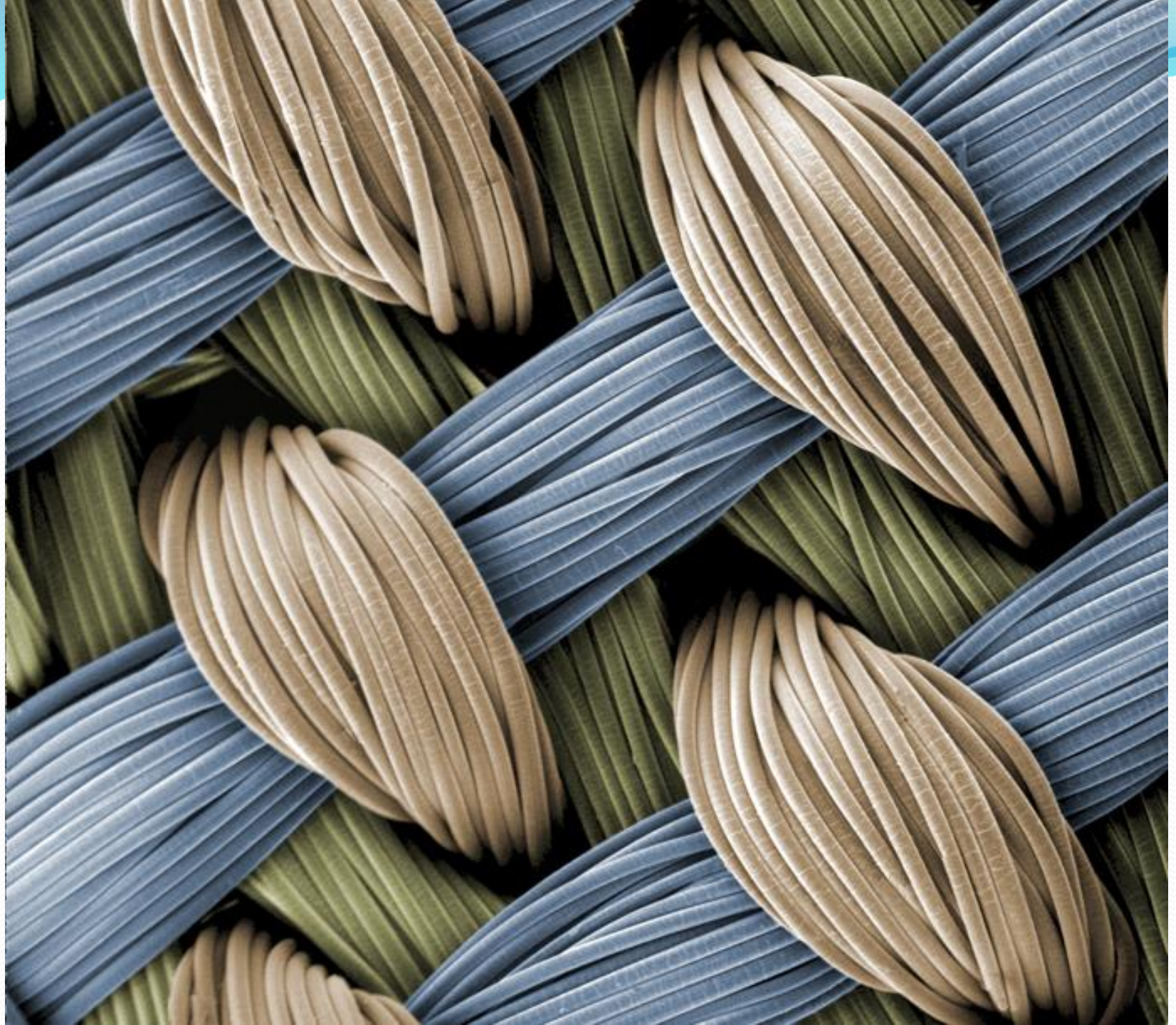
5. **3D printing**

1: Nanofiber self-assembly

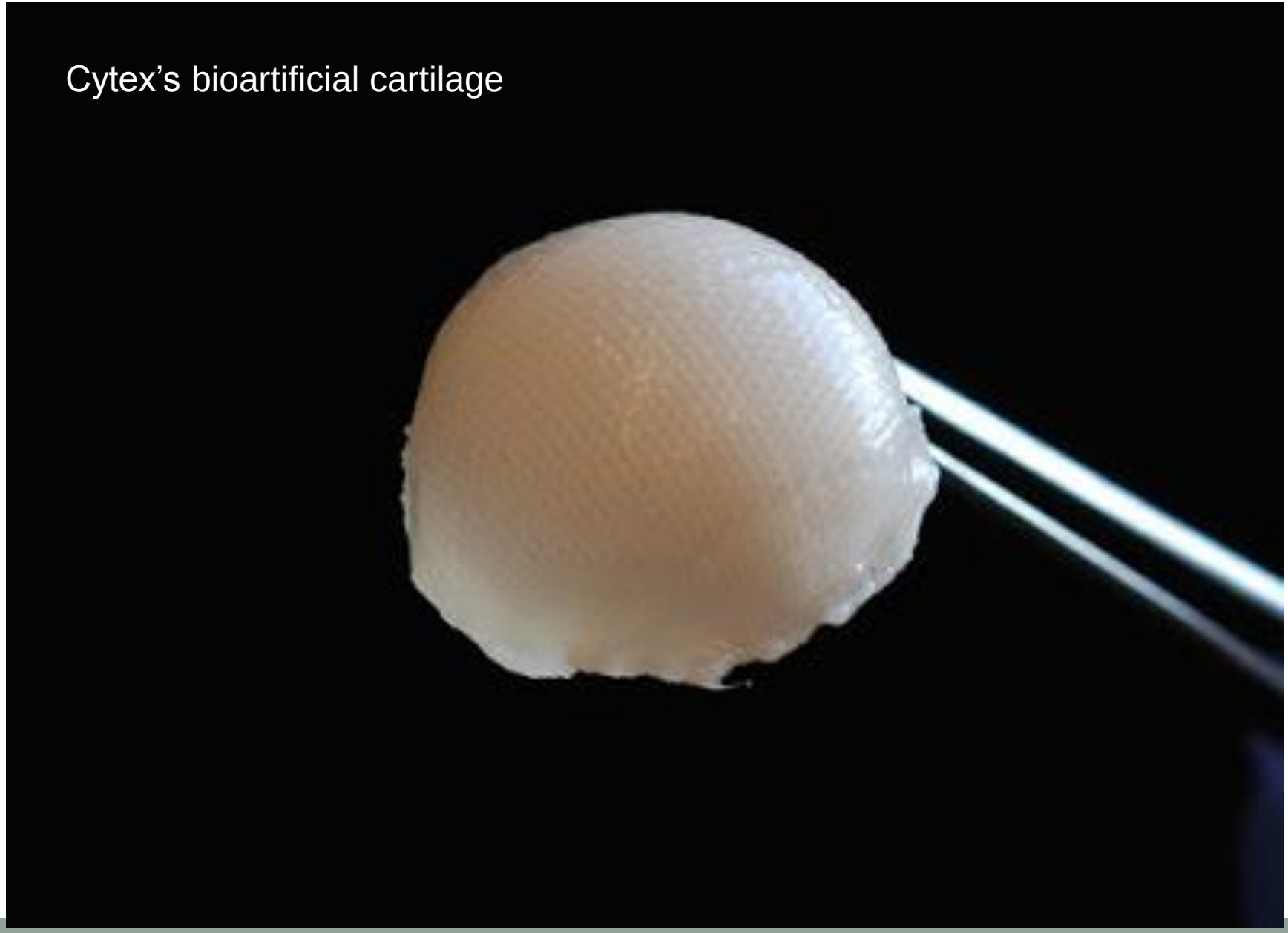


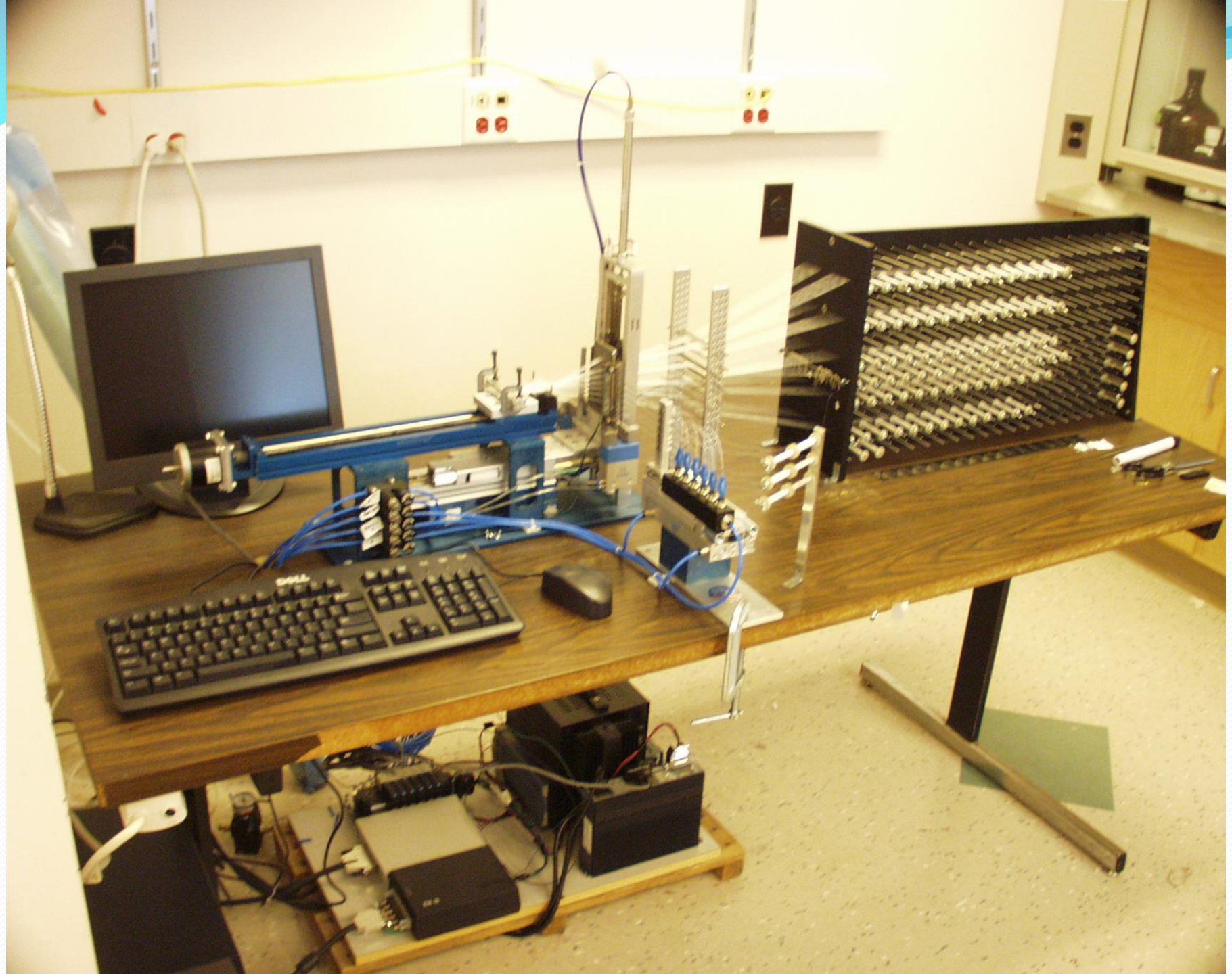
3-D weaving of genetically engineered stem cells is used to grow a living hip replacement

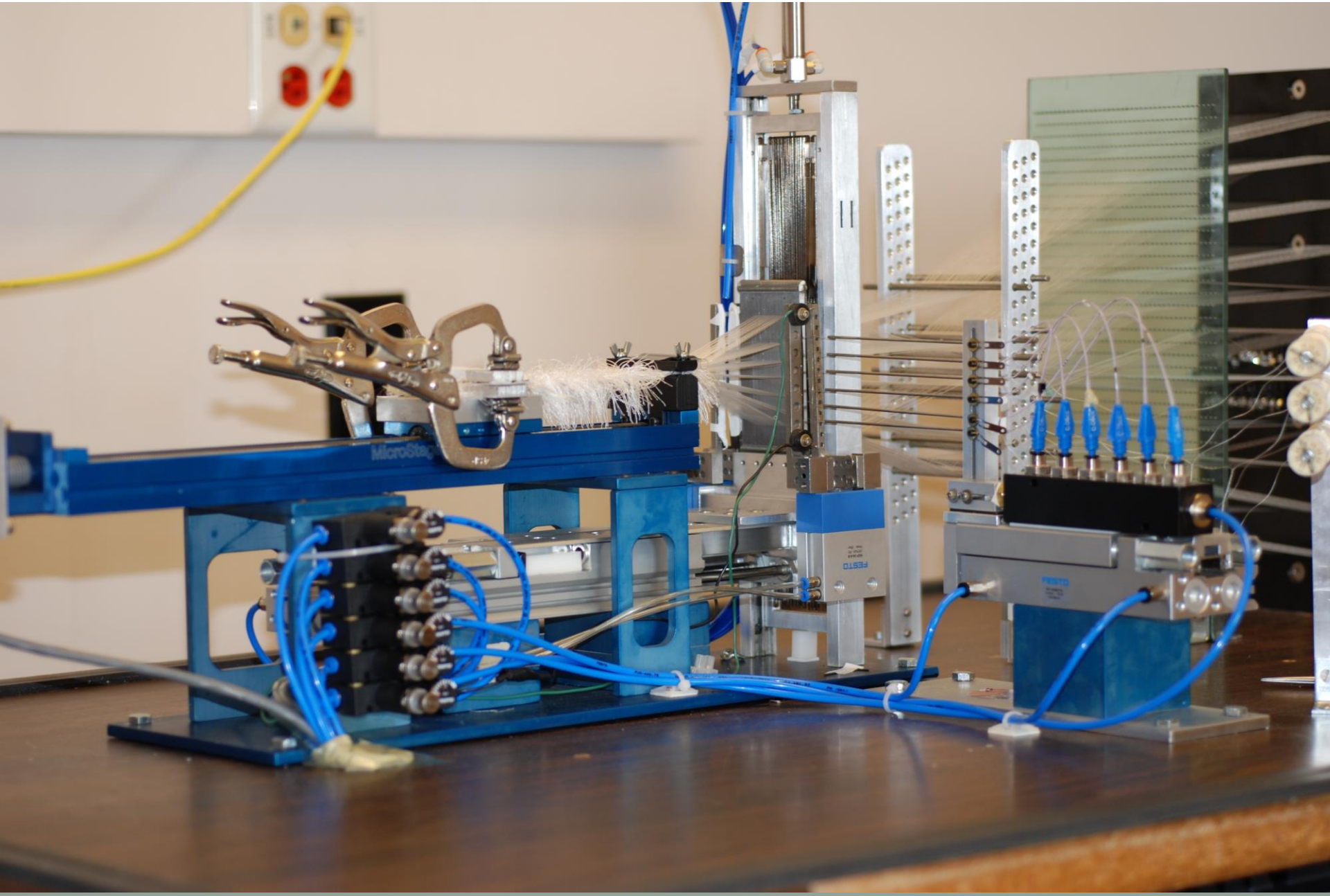
brian wang | July 20, 2016 |

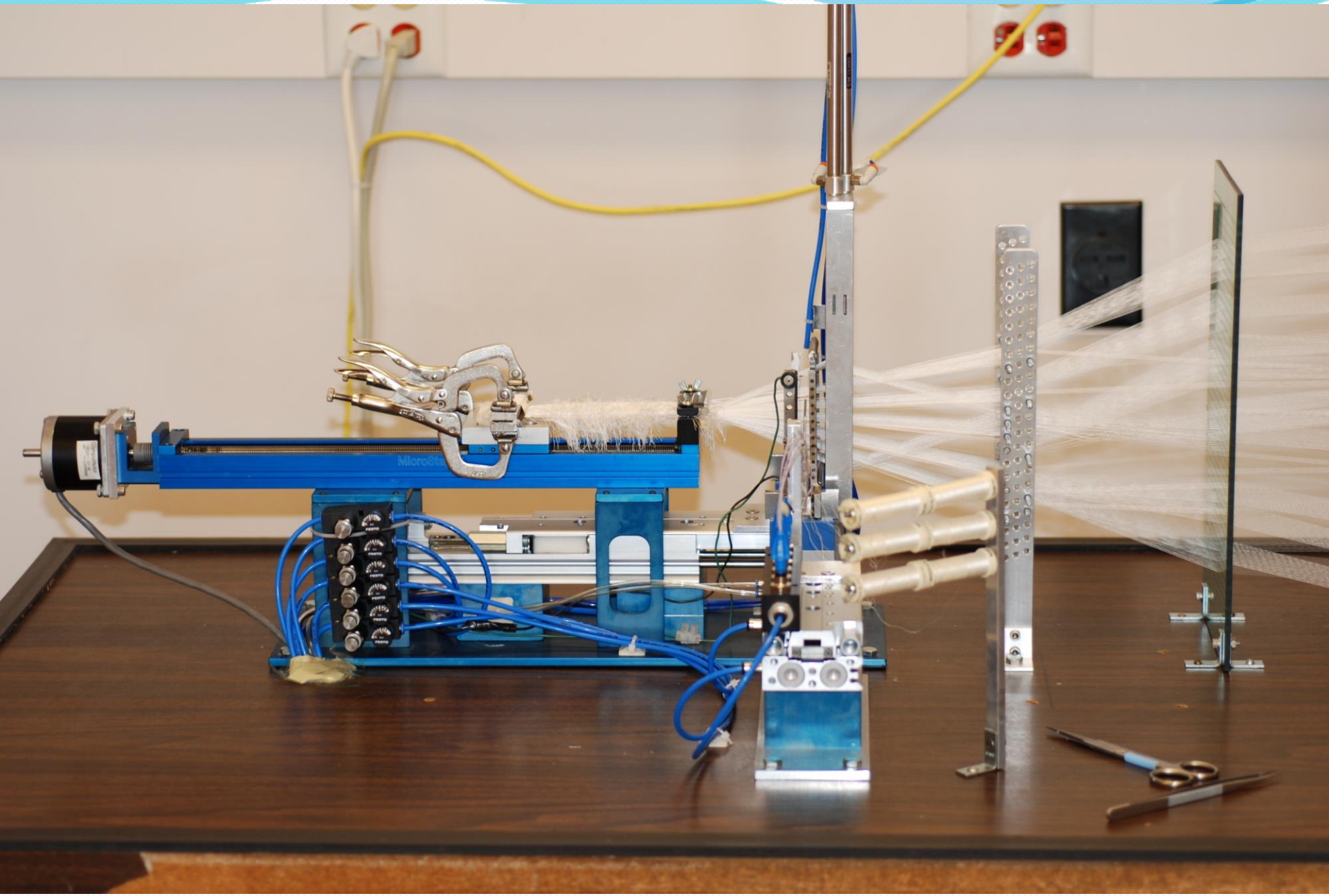


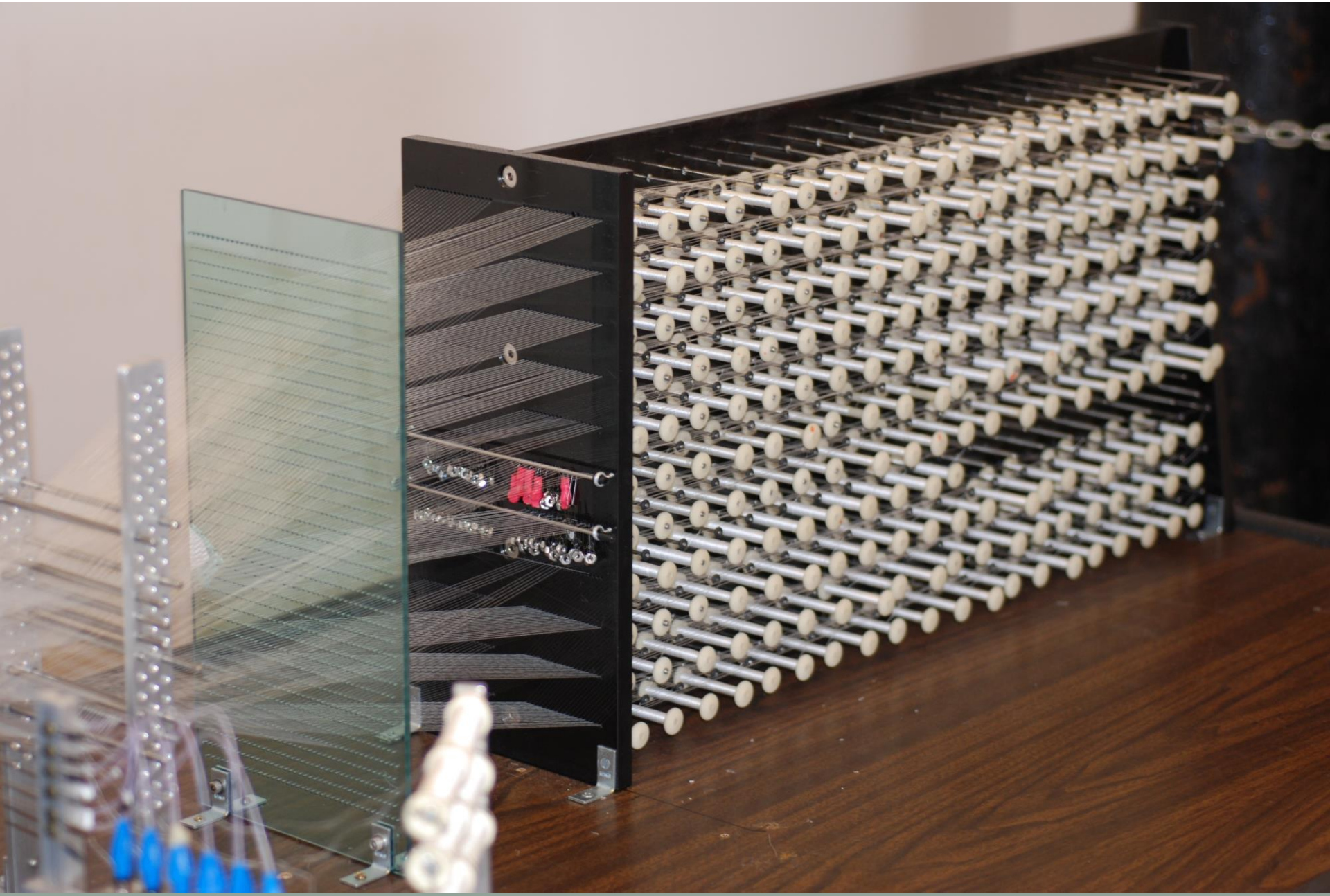
Cytex’s bioartificial cartilage





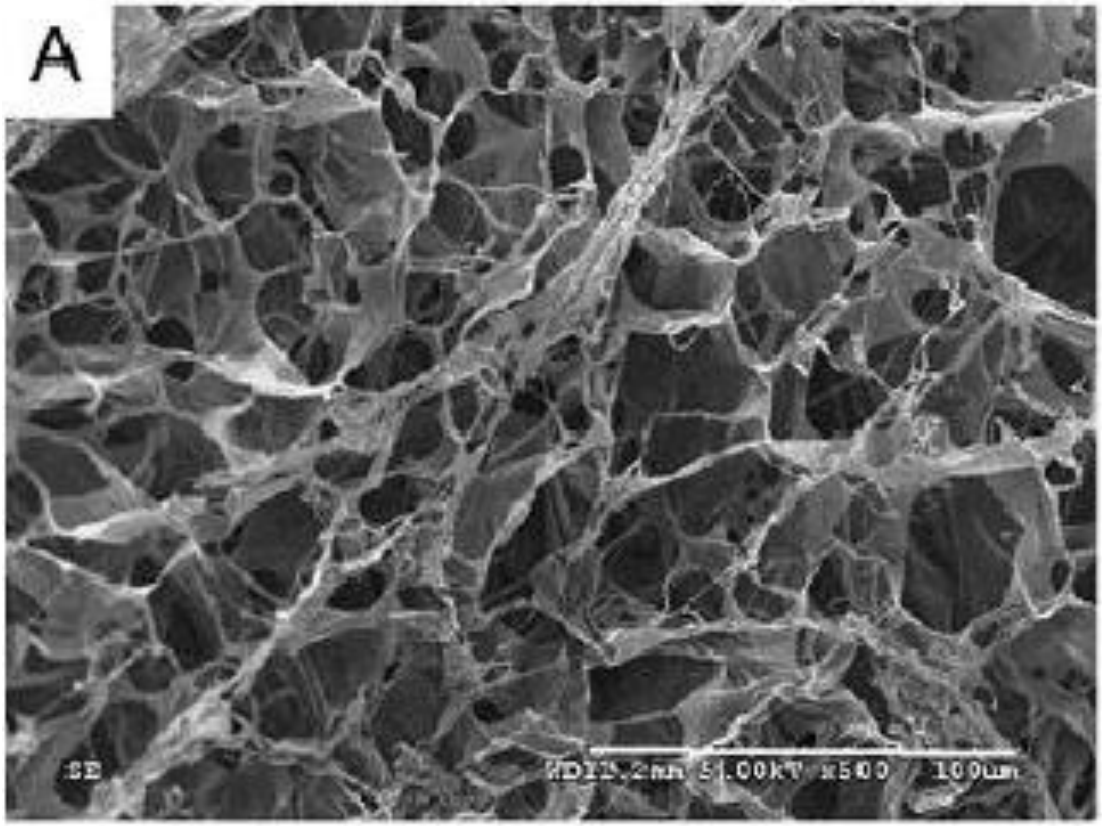






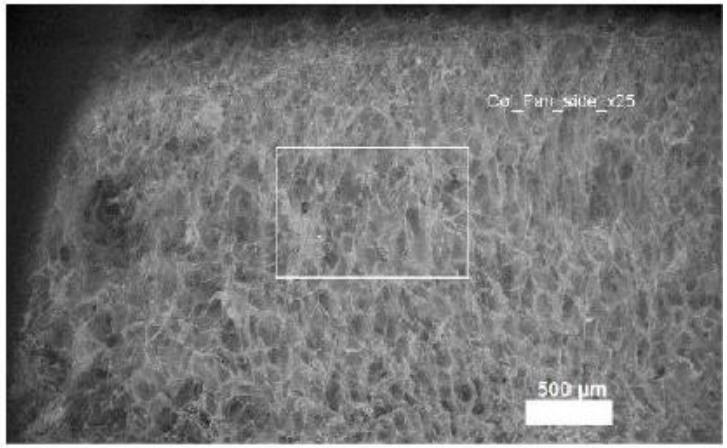
PLAY FRANK’s VIDEO

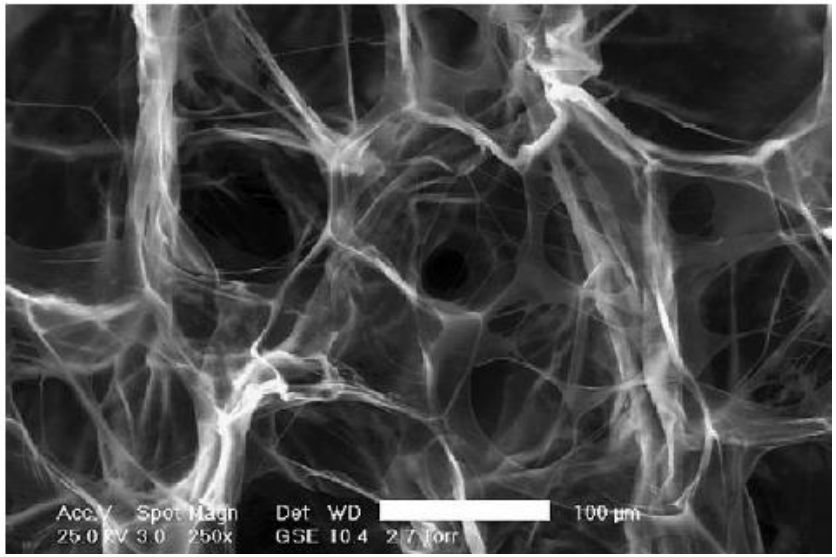
3: Freeze Drying Technology



Porous Collagen Scaffolds

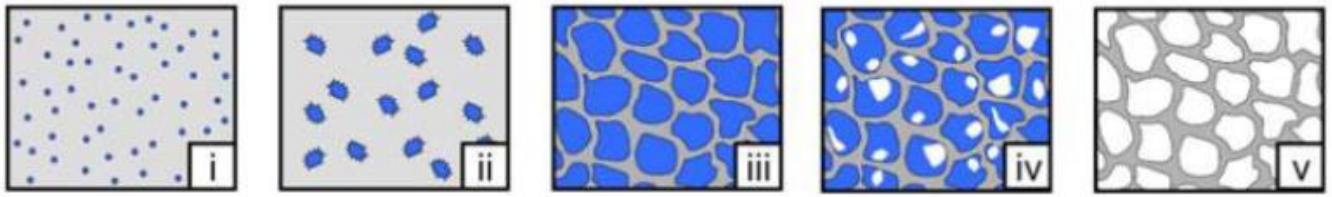
- Aka. “artificial skin”
- Key properties
  - Type I microfibrillar collagen
  - 0.5 - 5% solid fraction
  - Aprox. 100 μm pore diameter
  - Aprox 3 weeks *in vivo* half life



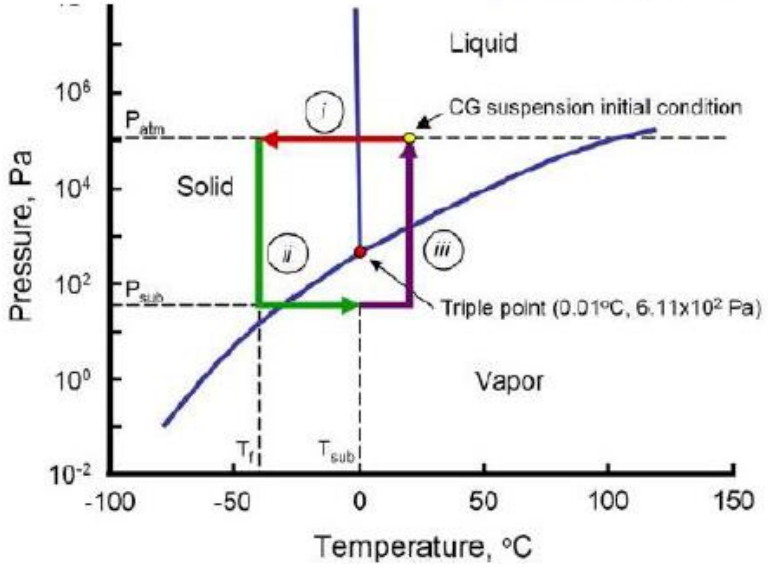


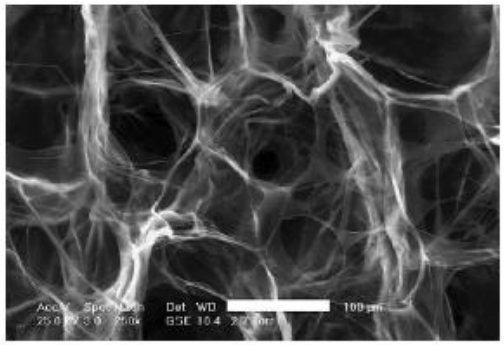
Porous Collagen Scaffold Fabrication

- Freeze-drying



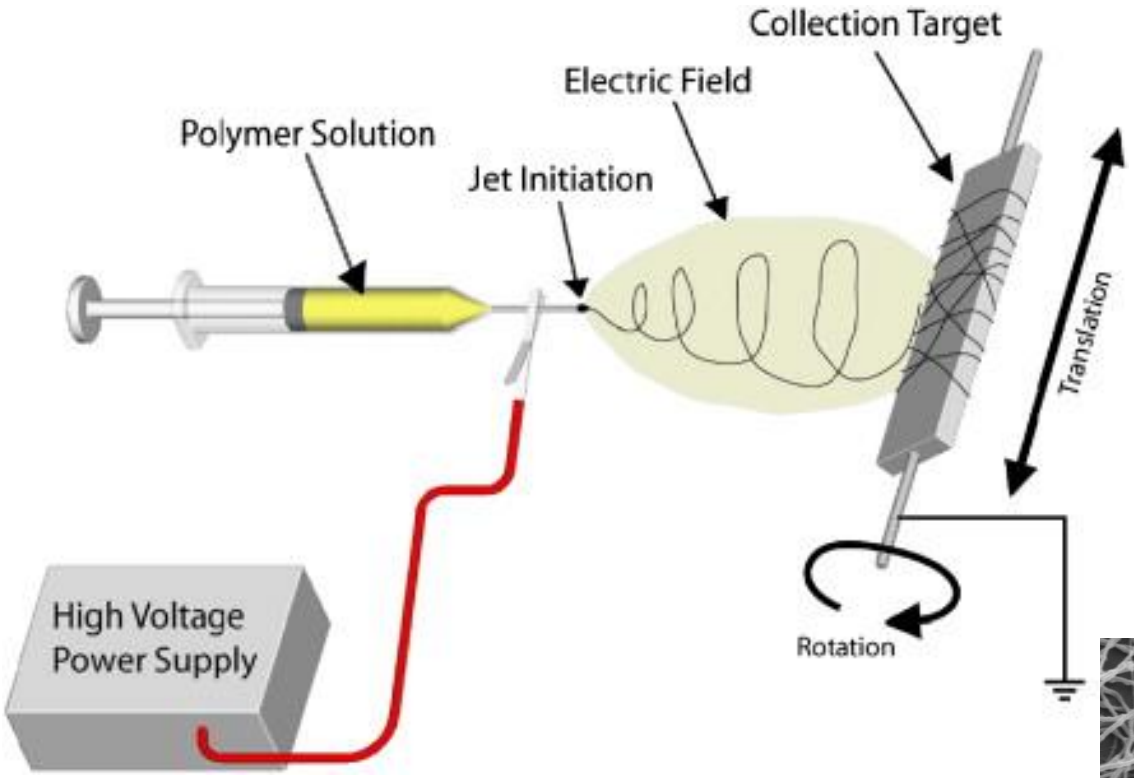
CG suspension
Pore
Ice crystals
Solid CG content (scaffold)

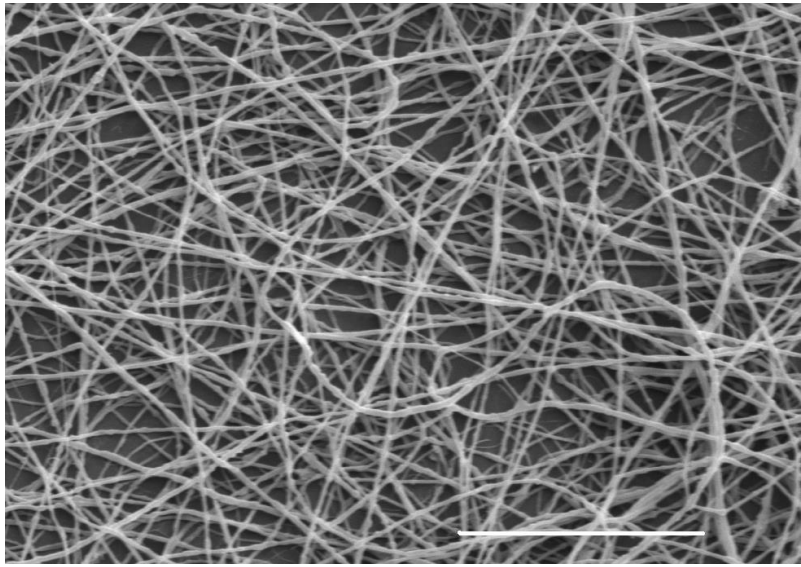




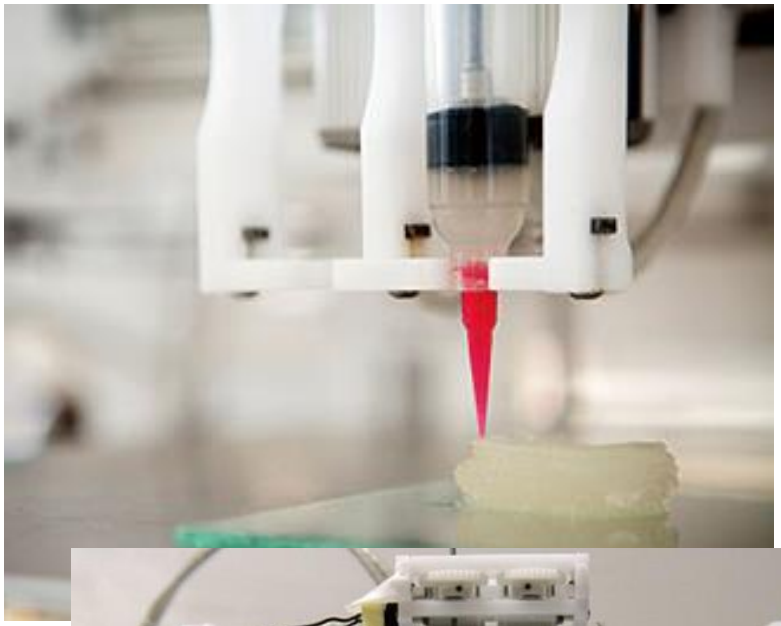
Images by Harley 2012

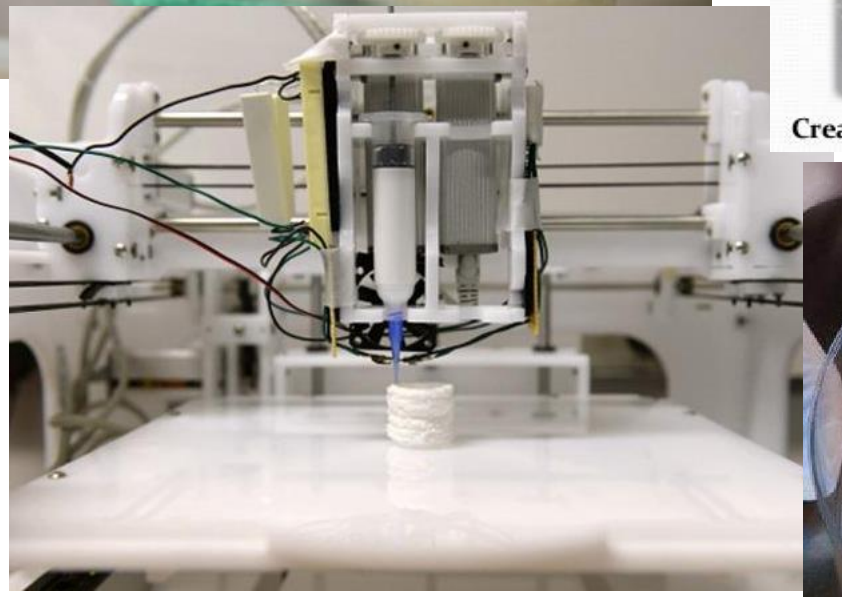
4: electrospinning

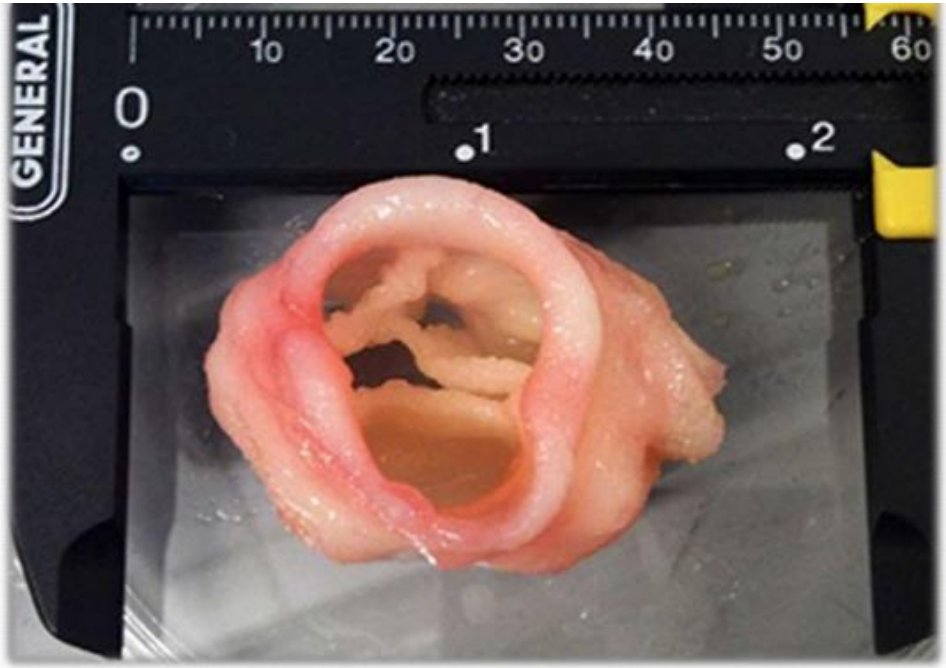




5: 3D printing

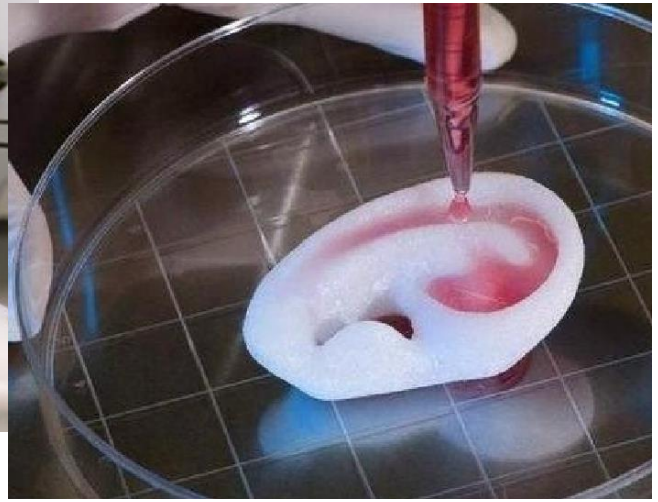






Creating Valve Tissue Using 3-D Bioprinting

Source: *Butcher et al., 2012*



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Differentiated , Stem Cells

SCAFFOLDS

Synthetic (PLA, PGA, etc)

Natural polymers (Alginate, Collagen, HA, etc)

BIOACTIVE ENVIRONMENT

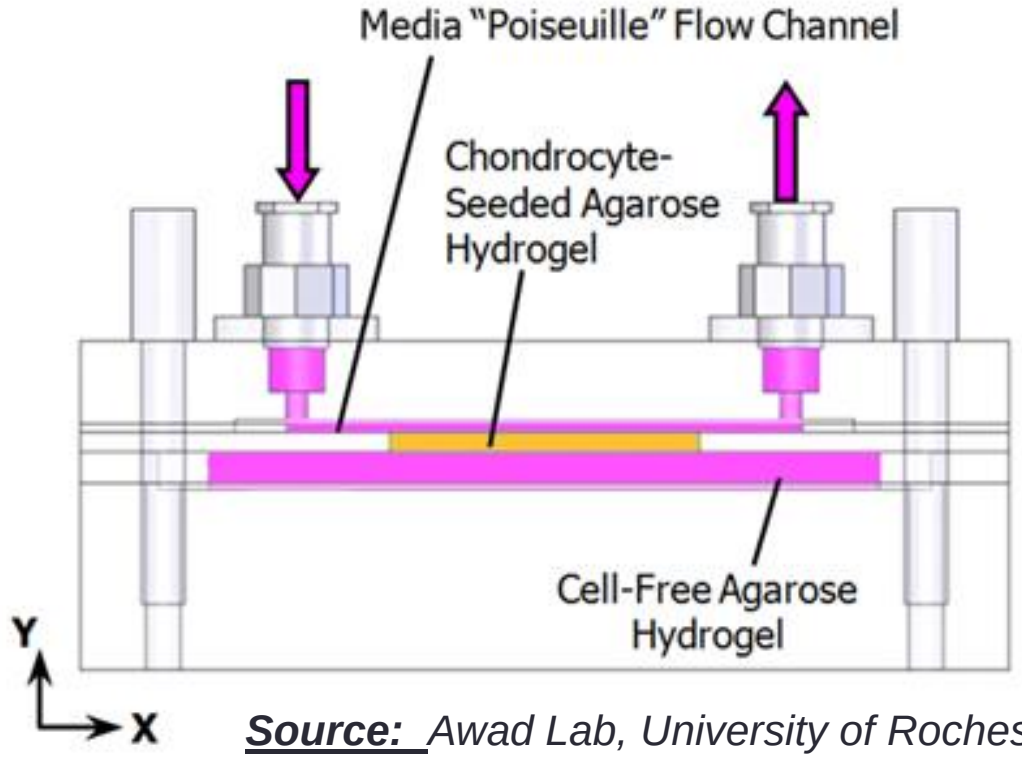
Growth factors , Hormones Cytokines , Mechanical environment

3. Bioreactors

- A tissue engineering **bioreactor** can be defined as **a device** that uses **mechanical means** to influence **biological processes** (Darling and Athanasiou, 2003).

- Bioreactors can be used to aid in the in-vitro development of new tissue by providing biochemical and physical regulatory signals to cells and encouraging them to undergo differentiation and/or to produce ECM prior to in-vivo implantation (Darling and Athanasiou, 2003).

Video showing a bioreactor for muscle fibers:
https://www.youtube.com/watch?v=XmDeaP6n9vA



Source: *Awad Lab, University of Rochester*

3.1 Introduction to Bioreactors

- Cells do respond to mechanical stimulation and bioreactors can be used to apply such mechanical stimulation to cells. Such stimuli encourage cells to produce extracellular matrix (ECM) in a **shorter** time period and in a more **homogeneous** manner than would be the case with static culture.

- According to Carver, PGA scaffolds seeded with equine articular chondrocytes expressed significantly improved ECM protein levels when cultured under hydrostatic pressure in comparison with the same scaffolds cultured in static medium.

- Another important application of bioreactors is in cellular differentiation. Mechanical stimulation can be used to encourage stem cells down a particular path and hence provide the cell phenotype required.

3.1 Introduction to Bioreactors

- As well as providing mechanical stimulation, **bioreactors** can also be used to improve **cellular spatial distribution.**

- A heterogeneous cell distribution is a major obstacle to developing any 3-D tissue or organ in-vitro. Defects requiring tissue engineering solutions are typically many millimeters in size (Goldstein, et al., 2001).

- Scaffolds in such a size range are easily fabricated, however, problems arise when culturing cells on these scaffolds. Static culture conditions result in scaffolds with few cells in the centre of the construct (Cartmell, et al., 2003).

- It has been shown that despite homogeneous cell seeding, after long periods in culture, more cells are found on the periphery of constructs (Cartmell, et al., 2003), leading to peripheral encapsulation which hinders nutrients and waste exchange from the centre, resulting in core degradation of TE constructs.

- Thus, bioreactors can be used in TE applications to overcome problems associated with traditional static culture conditions.

3.1 Introduction to Bioreactors

- According to Vunjak-Novakovic, bioreactors are designed to perform at least one of the following five functions:

1. Provide a spatially uniform cell distribution
2. Maintain the desired concentration of gases and nutrients in culture medium
3. Facilitate mass transport to the tissue
4. Expose the construct to physical stimuli
5. Provide information about the formation of 3-D tissue

3.2 Bioreactor Design Requirements

- The design of a bioreactor should be as simple as possible e.g. avoiding the introduction of machined recesses which could become breeding grounds for micro-organisms.

- Simplicity in design should also mean that the bioreactor is quick to assemble and disassemble. Apart from being more efficient, this ensures that cell-seeded constructs inserted into the bioreactor are out of the incubator for the maximum amount of time possible.

- The material selection is very important as it is vital to ensure that the materials used to create the bioreactor do not elicit any adverse reaction from the cultured tissue.

- Any material which is in contact with media must be biocompatible or bioinert. This eliminates the use of the most metals except for stainless steel and titanium.

3.2 Bioreactor Design Requirements

- Materials must be usable at 37^{o} C in a humid atmosphere. They must be able to be sterilized if they are to be re-used.

- Materials with different properties are needed for various components in the bioreactor. For example, transparent materials can be of benefit in allowing the construct to be monitored in the bioreactor during culture while flexible tubing can help with assembly of the bioreactor.

- If various parameters such as pH, nutrient concentration or oxygen levels are to be monitored, these sensors should be incorporated into the design.

- The forces needed for cellular stimulation are very small so it is important to ensure that the pump motor has the capability to apply small forces accurately.

3.3 Types of Bioreactors

- Numerous types of bioreactors are used in various tissue engineering applications such as:

1. Spinner Flask Bioreactor
2. Rotating Wall Bioreactor
3. Compression Bioreactor
4. Strain Bioreactor
5. Hydrostatic Pressure Bioreactors
6. Flow Perfusion Bioreactor

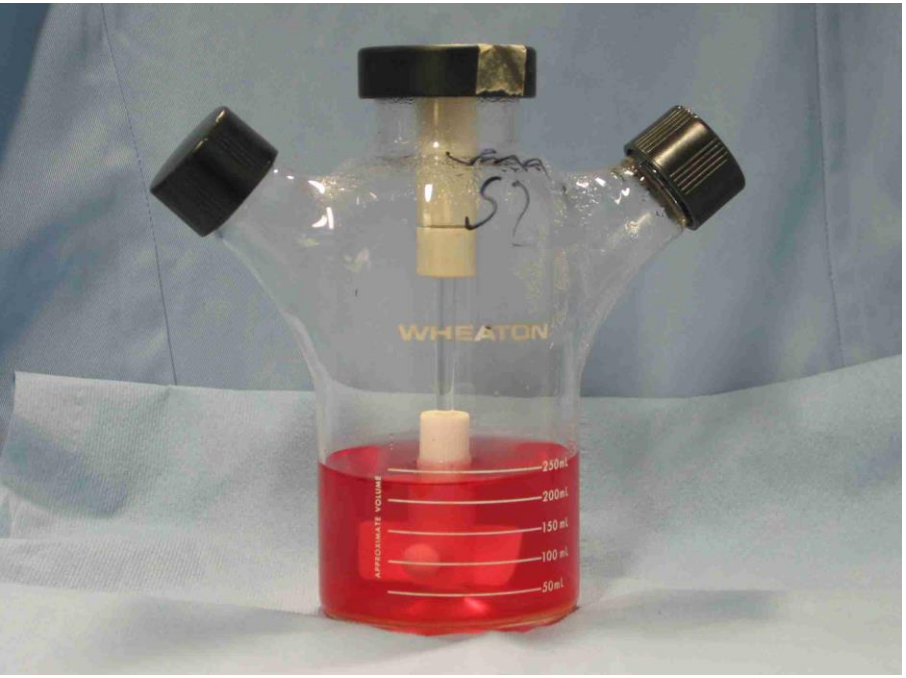
3.3.1 Spinner Flask Bioreactor

- A spinner flask bioreactor induces mixing of oxygen and nutrients throughout the medium and reduces the concentration boundary layer at the construct surface (Goldstein. et al., 2001).

- Scaffolds are suspended at the end of needles in a flask of culture media. A magnetic stirrer mixes the media and the scaffolds are fixed in place with respect to the moving fluid (Goldstein. et al., 2001).

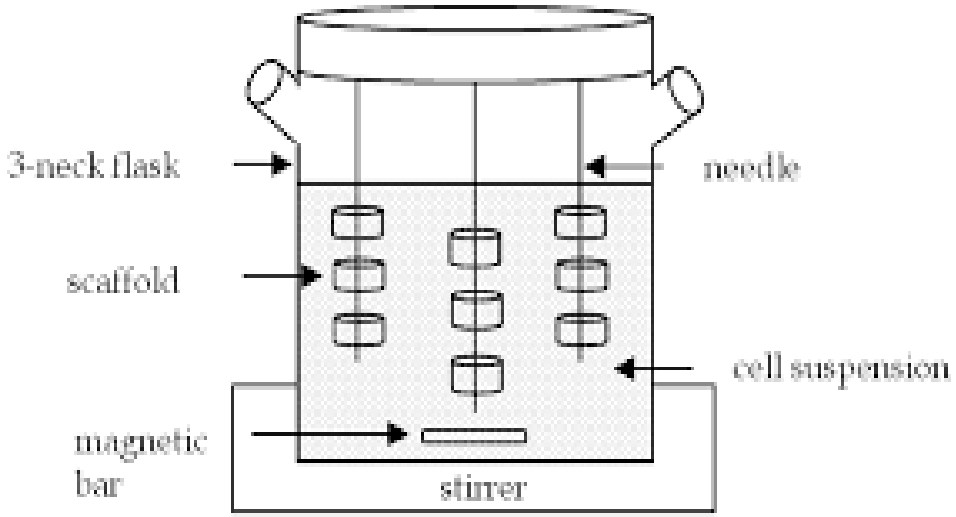
- Typically, spinner flasks are around 120mL in volume and run at 50-80rpm and 50% of the medium used in them is changed every two days (Vunjak-Novakovic, 2000).

3.3.1 Spinner Flask Bioreactor



Video showing spinner flask bioreactors inside an incubator: https://www.youtube.com/watch?v=IwKDynxNAKE

Source: Max Planck Institute, Monitoring, Design and Optimization of Bioprocesses

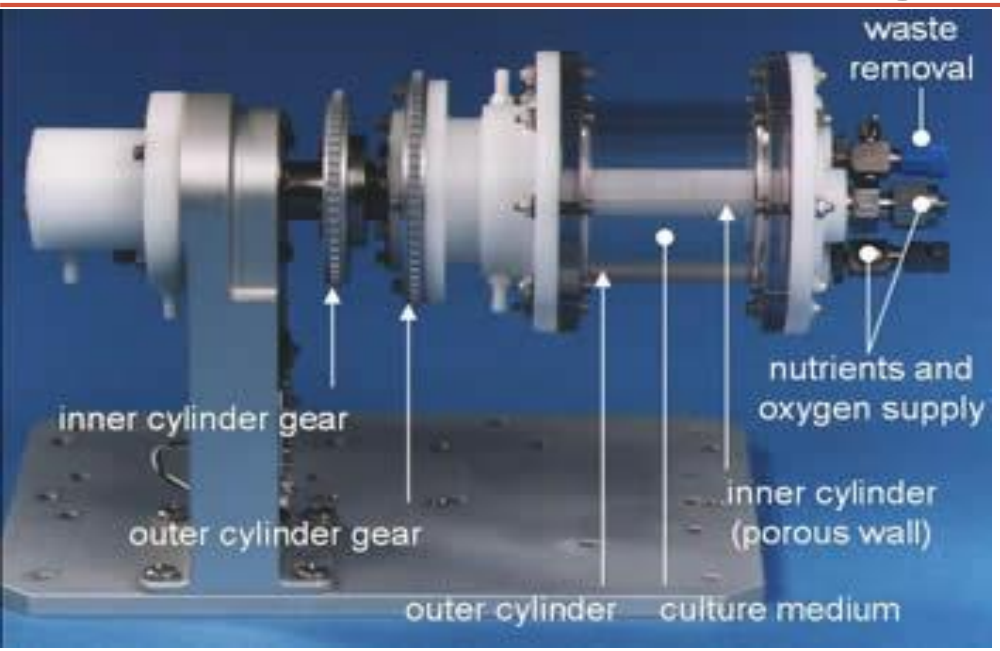


Source: Partak, et al., Intech

3.3.2 Rotating Wall Bioreactor

- The rotating wall bioreactor was developed by NASA (Schwarz et al., 1992).
- Scaffolds are free to move in media in a vessel. A rotating wall vessel bioreactor consists of a cylindrical chamber in which the outer wall, inner wall, or both are capable of rotating at a constant angular speed. The vessel wall is then rotated at a speed such that a balance is reached between the downward gravitational force and the upward hydrodynamic drag force acting on each scaffold (Freed et al., 1997).
- The wall of the vessel rotates, providing an upward hydrodynamic drag force that balances with the downward gravitational force, resulting in the scaffold remaining suspended in the media (Freed et al., 1997).
- Media can be exchanged by stopping the rotation temporarily or by adding a fluid pump whereby media is constantly pumped through the vessel (Freed et al., 1997).
- Gas exchange occurs through a gas exchange membrane and the bioreactor is rotated at speeds of 15-30 rpm (Freed et al., 1997).

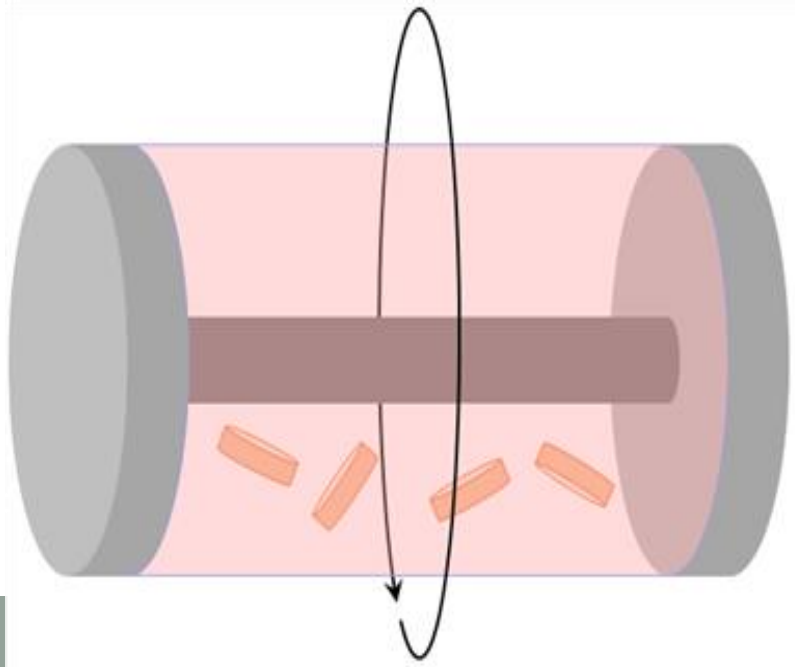
3.3.2 Rotating Wall Bioreactor

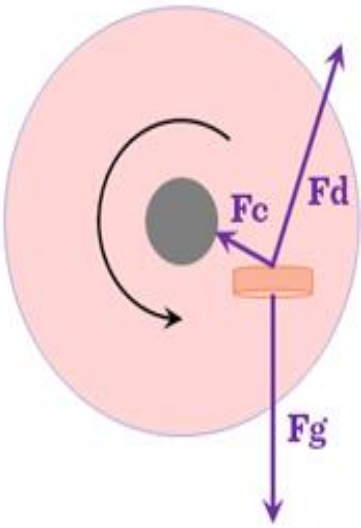


Video showing a rotating wall bioreactor:
https://www.youtube.com/watch?v=pn_pqMa23Nw

Source: Bartis et al., 2011

Source: Haj et al., 2015





3.3.3 Compression Bioreactors

- This class of bioreactors is generally used in cartilage engineering and can be designed so that both static and dynamic loading can be applied (Darling and Athanasiou, 2003).

- In general, compression bioreactors consist of a motor, a system providing linear motion and a controlling mechanism used to provide displacements of different magnitudes and frequencies. A signal generator can be used to control the system including load cells while transformers can be used to measure the load response and imposed displacement (Huang, et al., 2004).

- The load can be transferred to the cell-seeded constructs *via* flat platents which distribute the load evenly (Thorpe, et al., 2008).

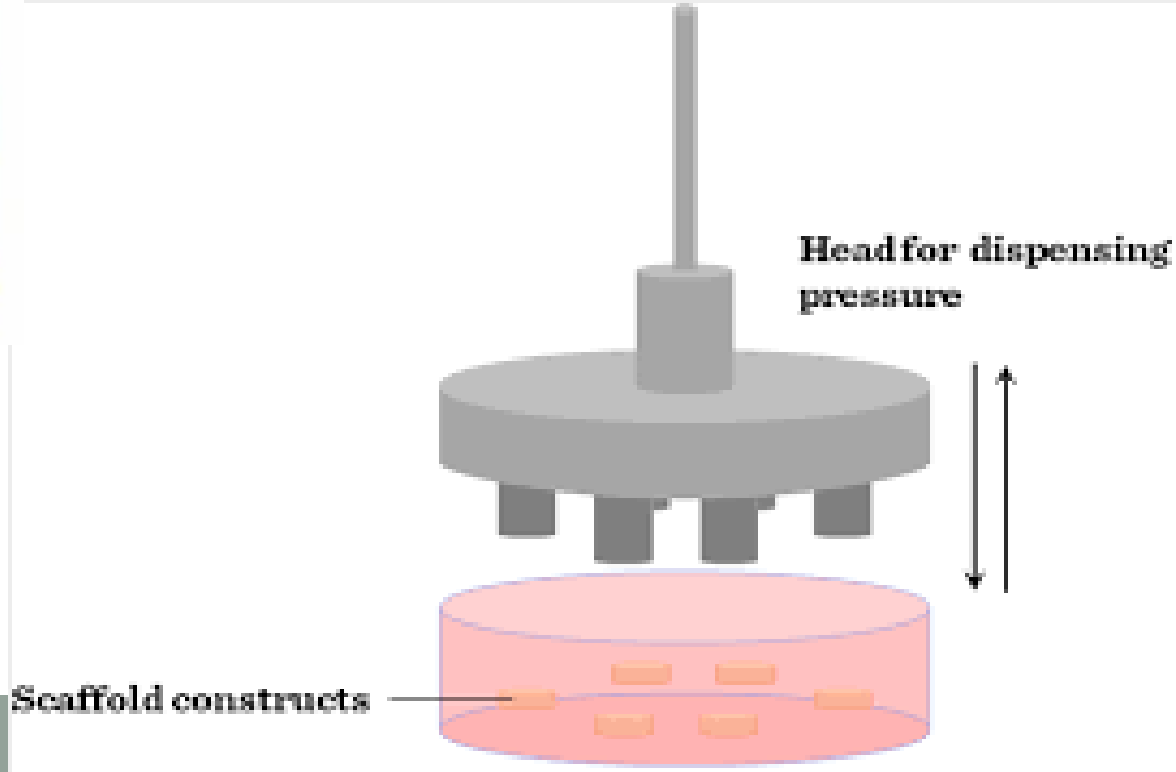
3.3.3 Compression Bioreactors

Source: McHenry, 2005

Video showing a compression bioreactor:

https://www.youtube.com/watch?v=O36YYp0ZIoU

Source: Bartis et al., 2011



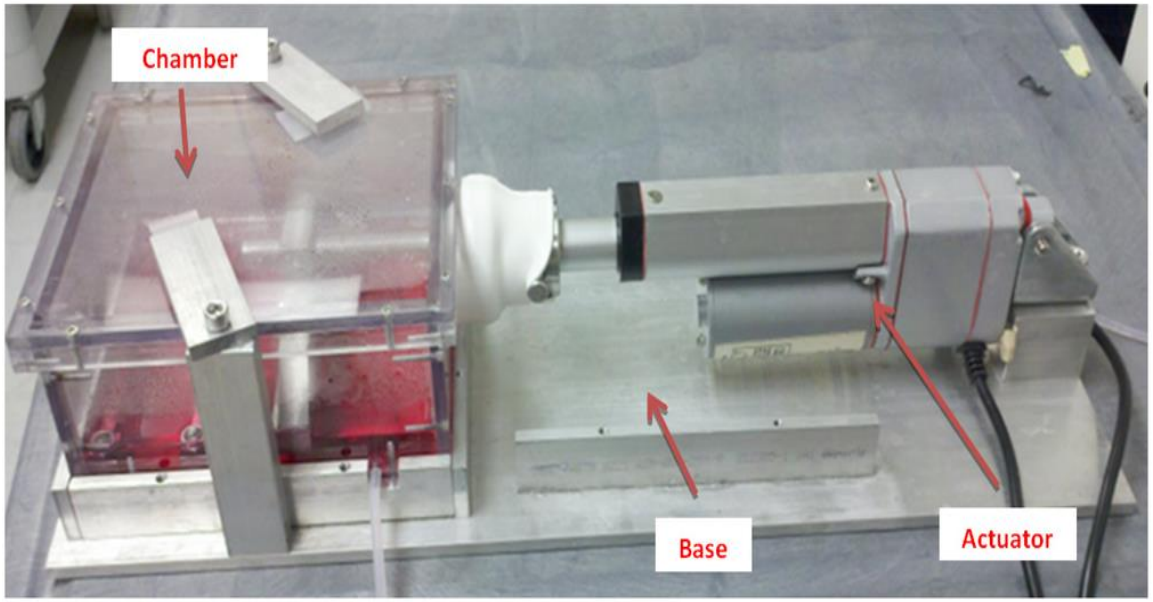
3.3.4 Strain Bioreactors

- Strain bioreactors have been used in an attempt to engineer a number of different types of tissues including **tendon, ligament, bone, cartilage and cardiovascular tissue.**

- Some designs are very similar to compression bioreactors, only differing in the way the force is transferred to the construct. Instead of flat platens as in a compression bioreactor, a way of clamping the scaffold into the device is needed so that a tensile force can be applied.

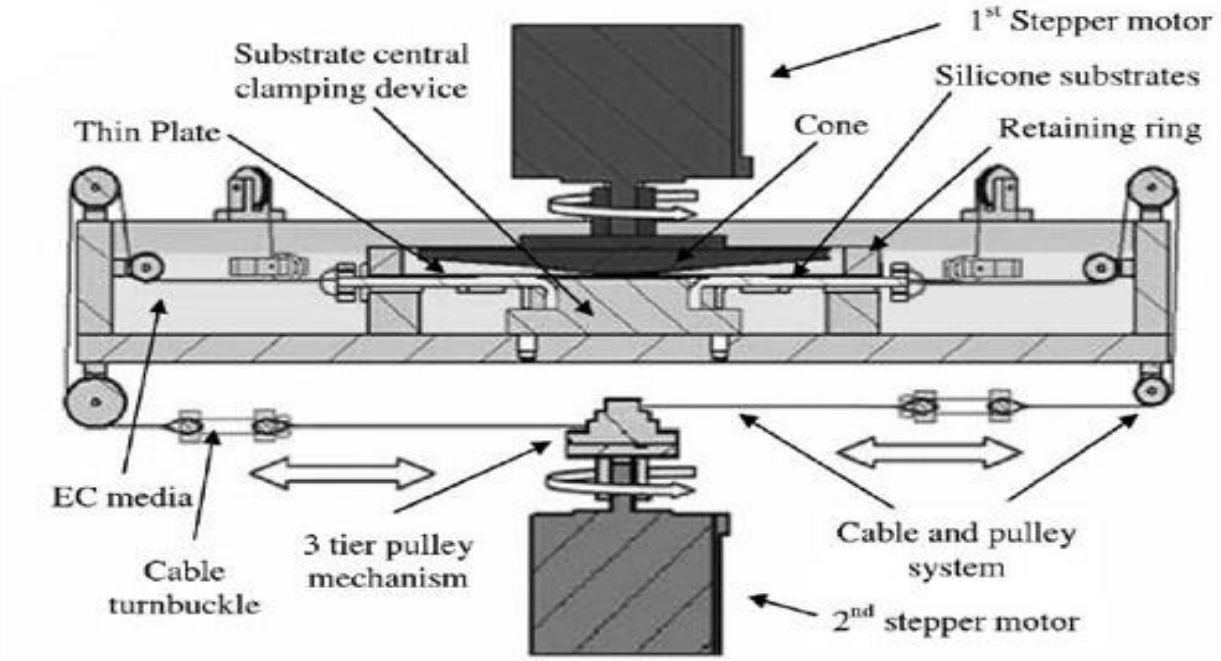
- Tensile strain has been used to differentiate MSC along the chondrogenic lineage. A multistation bioreactor was used in which cell-seeded collagen glycosaminoglycans scaffolds were clamped and loaded in uniaxial tension (McMahon, et al., 2008).

3.3.4 Strain Bioreactors



Uniaxial Strain Bioreactor for Engineered Ligaments : https://www.youtube.com/watch?v=FXCqcyNK0nM

Source: Goodhart et al., 2014



 Source: Breen, 2006

3.3.5 Hydrostatic Pressure Bioreactors

- In cartilage tissue engineering, HP bioreactors can be used to apply mechanical stimulus to cell-seeded constructs. Scaffolds are usually cultured statically and then moved to a hydrostatic chamber for a specified time for loading.

- HP bioreactors consist of a chamber which can withstand the pressure applied and a means of applying that pressure (Darling and Athanasiou, 2003).

- For example, a media-filled pressure chamber can be pressurised using a piston controlled by an actuator (Darling and Athanasiou, 2003). For sterility, the piston can apply pressure *via* an impermeable membrane so that the piston itself does not come in contact with the culture media.

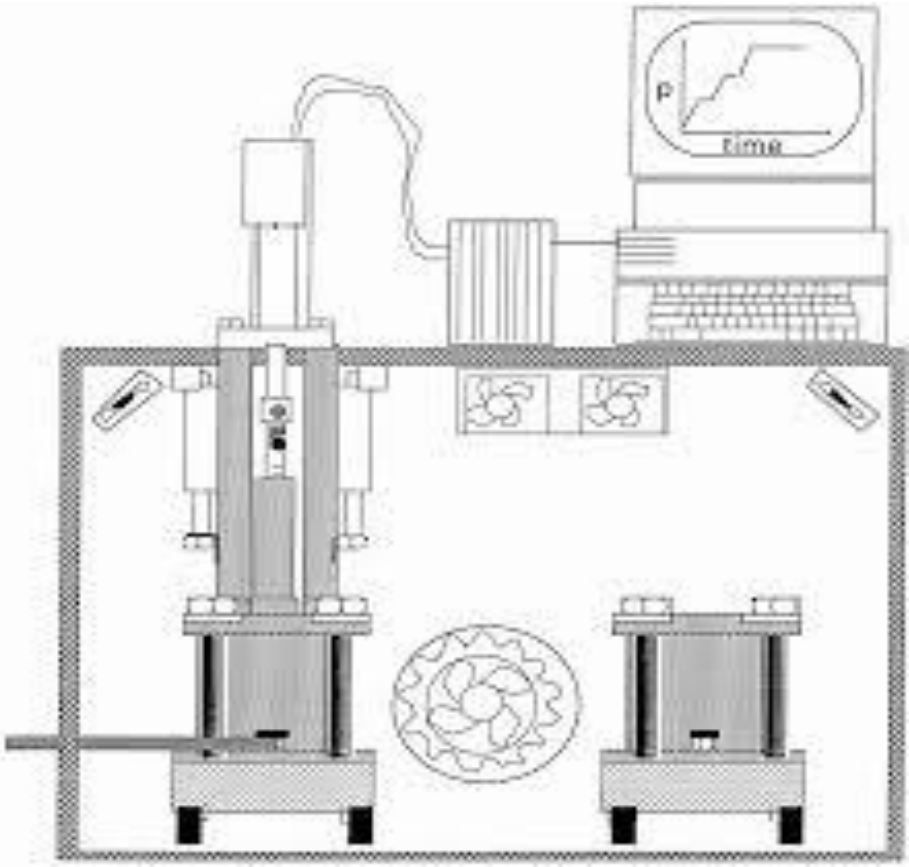
- Variations on this design include a water-filled pressure chamber which pressurises a media media-filled chamber *via* an impermeable film and is controlled using a variable backpressure valve and an actuator (Watanabe, et al., 2005).

3.3.5 Hydrostatic Pressure Bioreactors

Source: Eric Lima, Cooper Union Engineering Faculty

Video Showing a HP bioreactor:

https://www.youtube.com/watch?v=7sOKS2YQDq4



Source: Darling and Athanasiou, 2004

3.3.6 Flow Perfusion Bioreactors

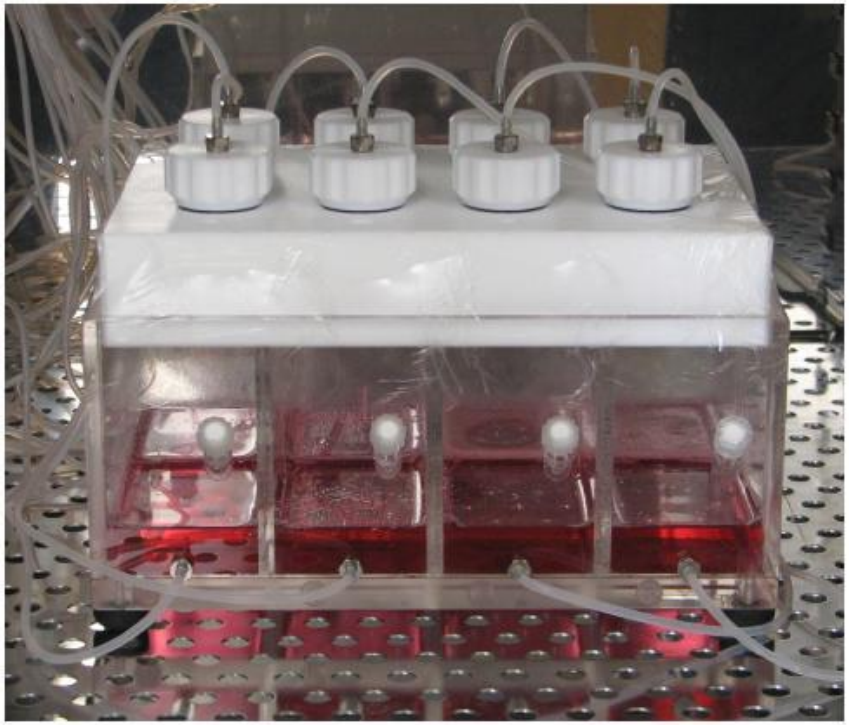
- Culture using flow perfusion bioreactors has been shown to provide more homogeneous cell distribution throughout scaffold.

- In comparisons between flow perfusion, spinner flask and rotating wall bioreactors, flow perfusion bioreactors have proved to be he best for fluid transport.

- Flow perfusion bioreactors generally consist of a pump and a scaffold chamber joined together by tubing. A fluid pump is used to force media flow through the cell-seeded scaffold. The scaffold is placed in a chamber that is designed to direct flow through the interior of the scaffold. The scaffold is kept in position across the flow path of the device and media is perfused through the scaffold, thus enhancing fluid transport.

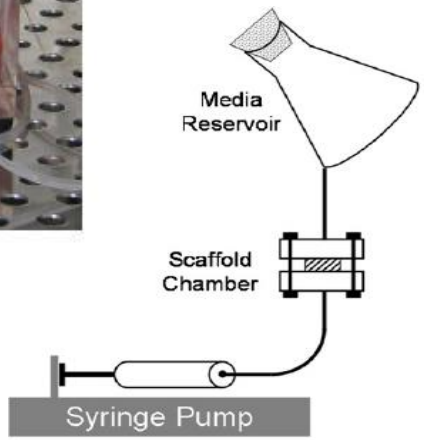
- Media can easily be replaced in the media reservoir

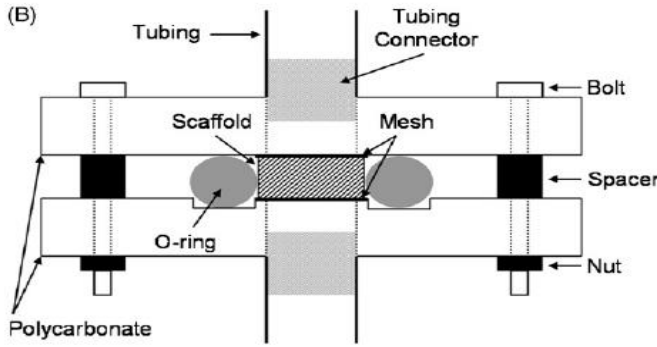
3.3.6 Flow Perfusion Bioreactors



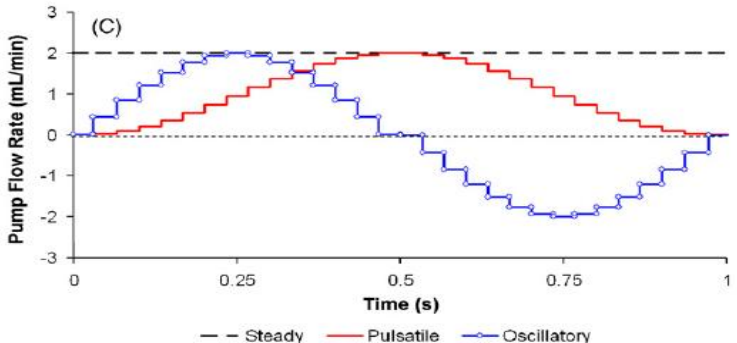
A 3-D Perfusion Bioreactor:

https://www.youtube.com/watch?v=XLC49jyj5EE



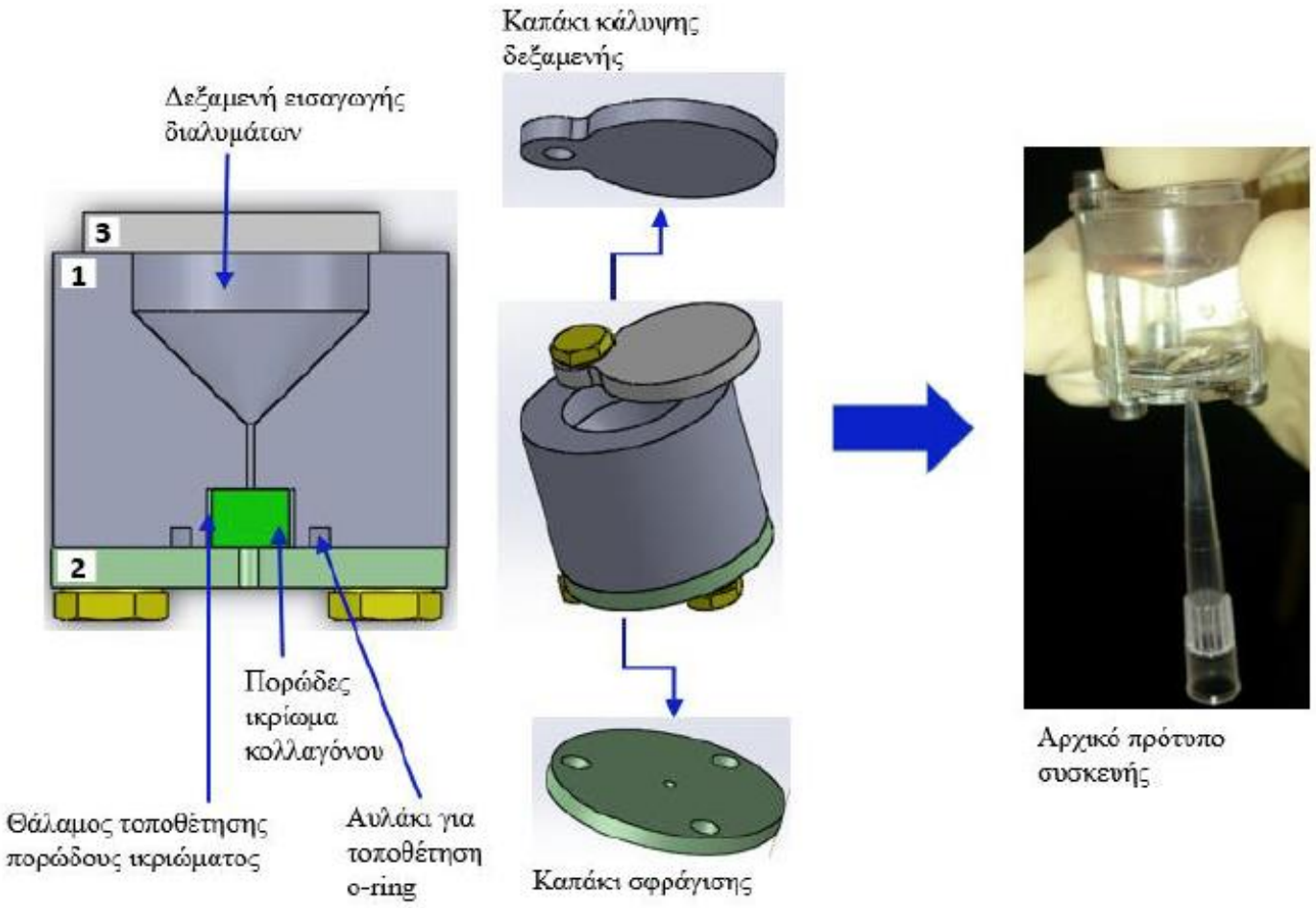


Source:
https://www.hindawi.com/journals/bmri/2009/873816/fig1/



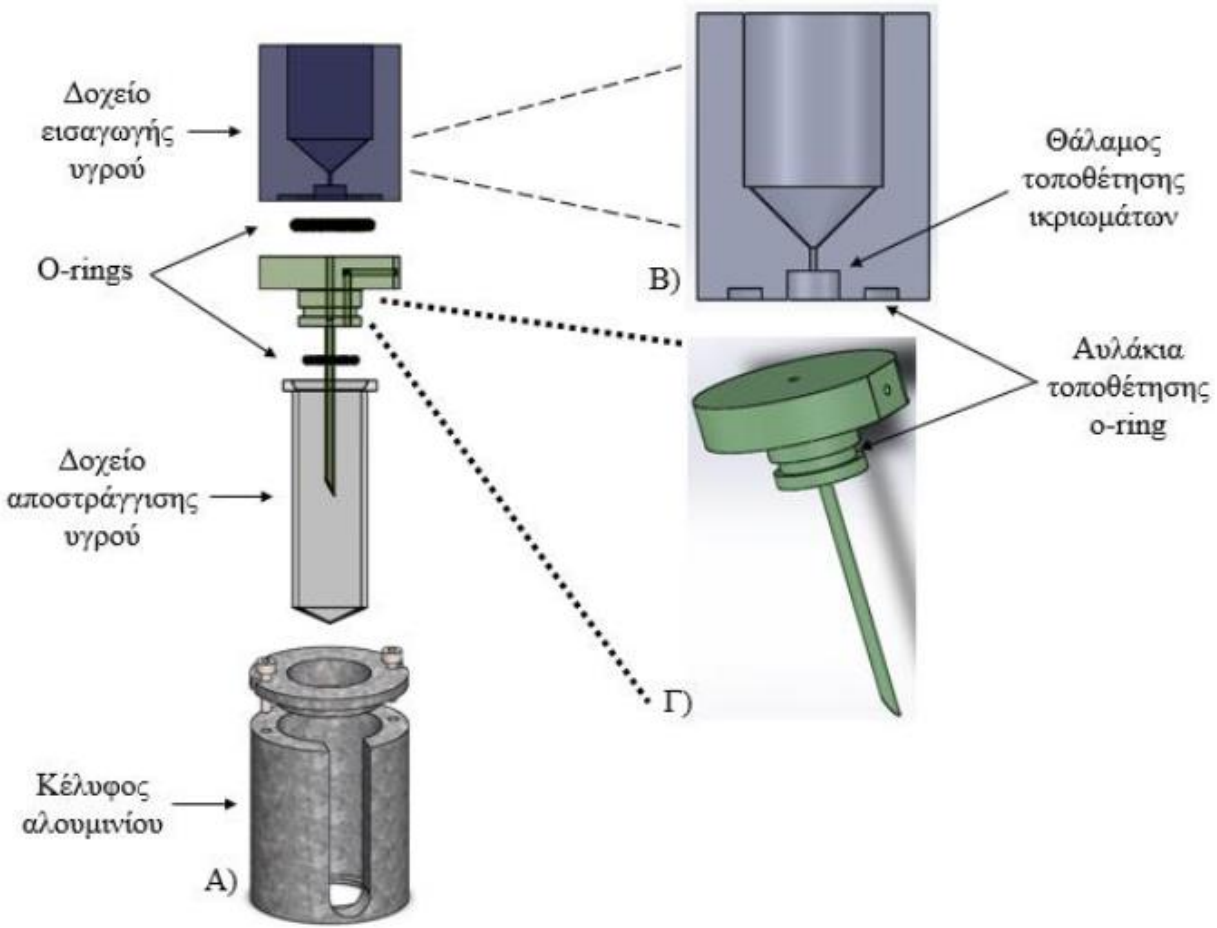
Source: Jaasma, et al., 2008

3.3.6 Flow Perfusion Bioreactors



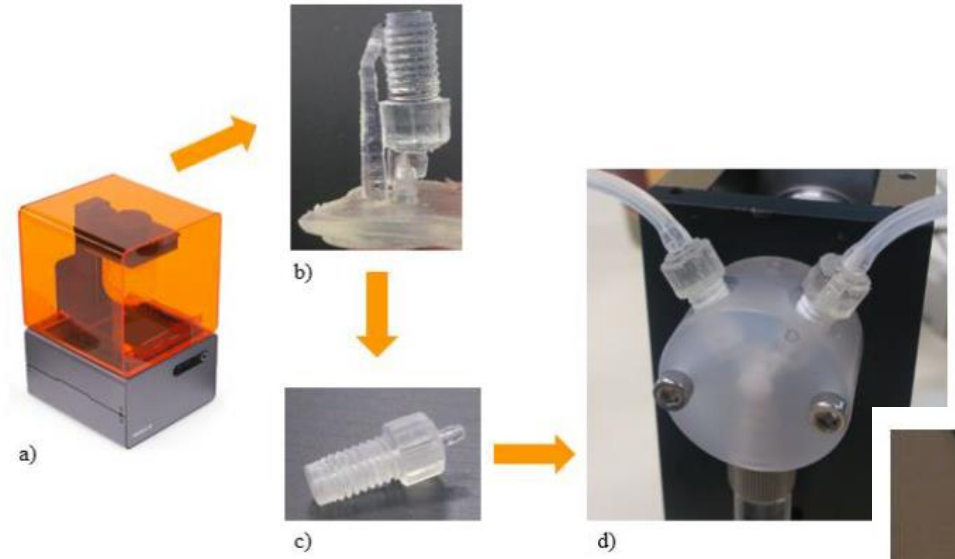
Εικόνα 10: Τρισδιάστατα μοντέλα CAD της συσκευής για καλλιέργεια κυττάρων σε πορώδη ικριώματα κολλαγόνου και αρχικό πρότυπο της συσκευής από PMMA

3.3.6 Flow Perfusion Bioreactors

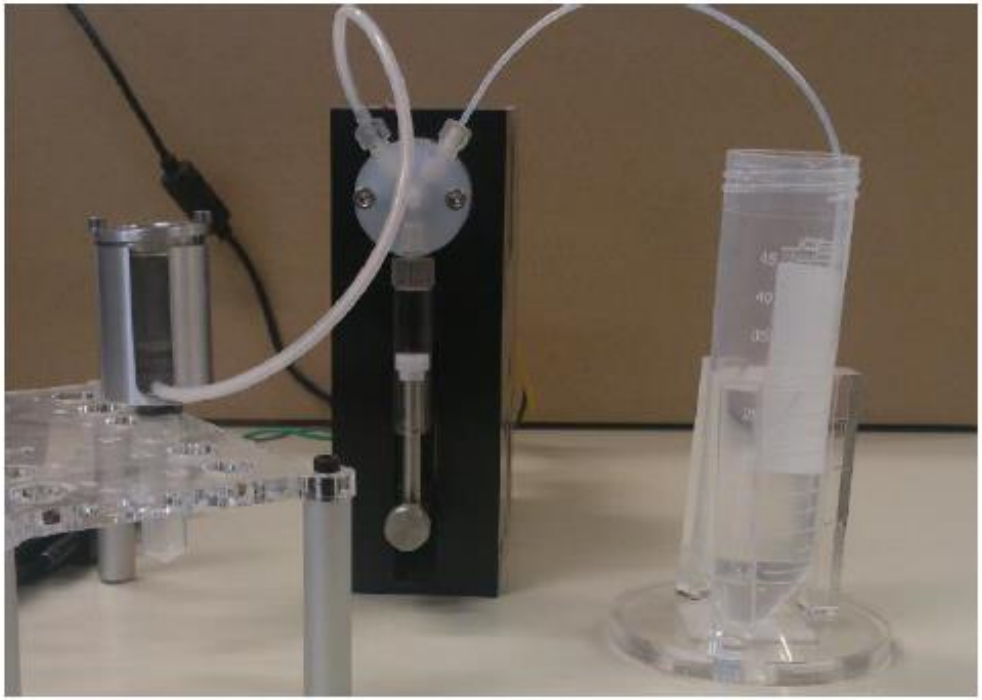


Εικόνα 11: Τρισδιάστατα μοντέλα CAD τροποποιημένου σχεδίου της συσκευής. Α) Κέλυφος αλουμινίου με καπάκι που πιέζει τα δύο κομμάτια της συσκευής Β, Γ με δύο βίδες Β) Θάλαμος τοποθέτησης πορώδους ικριώματος κολλαγόνου από PMMA, Γ) Τεμάχιο που βοηθά στη στεγανοποίηση της συσκευής και στη διασύνδεση της με το σύστημα της αντλίας από PMMA.

3.3.6 Flow Perfusion Bioreactors



Εικόνα 17: Στάδια δημιουργίας κατάλληλων προσαρμογών για το σύστημα της αντλί



Εικόνα 18: Συναρμολογημένη διάταξη συσκευής – αντλίας

The 4 components of Tissue Engineering

BIOREACTORS

Stress/strain controlled,
Shear Flow, Microgravity,
Perfusion, Regular incubator etc

CELLS

Autologous, Allogeneic,

Differentiated , Stem Cells

SCAFFOLDS

Synthetic (PLA, PGA, etc)

Natural polymers (Alginate,
Collagen, HA, etc)

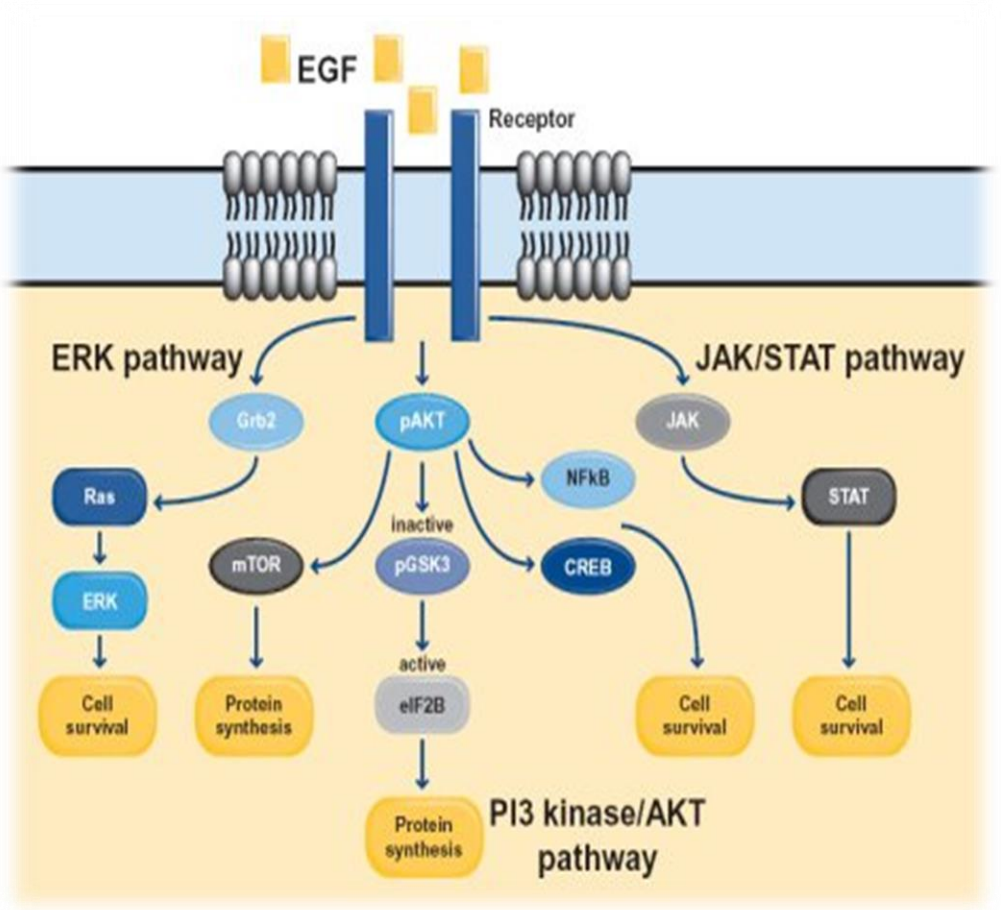
BIOACTIVE ENVIRONMENT

Growth factors , Hormones
Cytokines , Mechanical environment

4. Bioactive Environment

- A Bioactive environment in tissue engineering can be created with the utilization of:

1. Growth Factors
2. Hormones
3. Cytokines



Source: Lee, et al., 2010

4.1 Growth Factors

- Growth factors (GF) are protein molecules made by the body. They function to regulate cell devision and cell survival. GF can also be produced via genetic engineering in the lab and used in biological therapy.

- GF bind to receptors on the cell surface, with the result of activating cellular proliferation and/or differentiation. GFs are quite versatile, stimulating cellular devision in numerous different cell types.

- GFs secrete diffusible factors that are identified in the conditioned medium of cell cultures. Examples for GFs are: **EGF, FGF, NGF, PDGF, IGF , BMP, HGF, GDF and many others.**

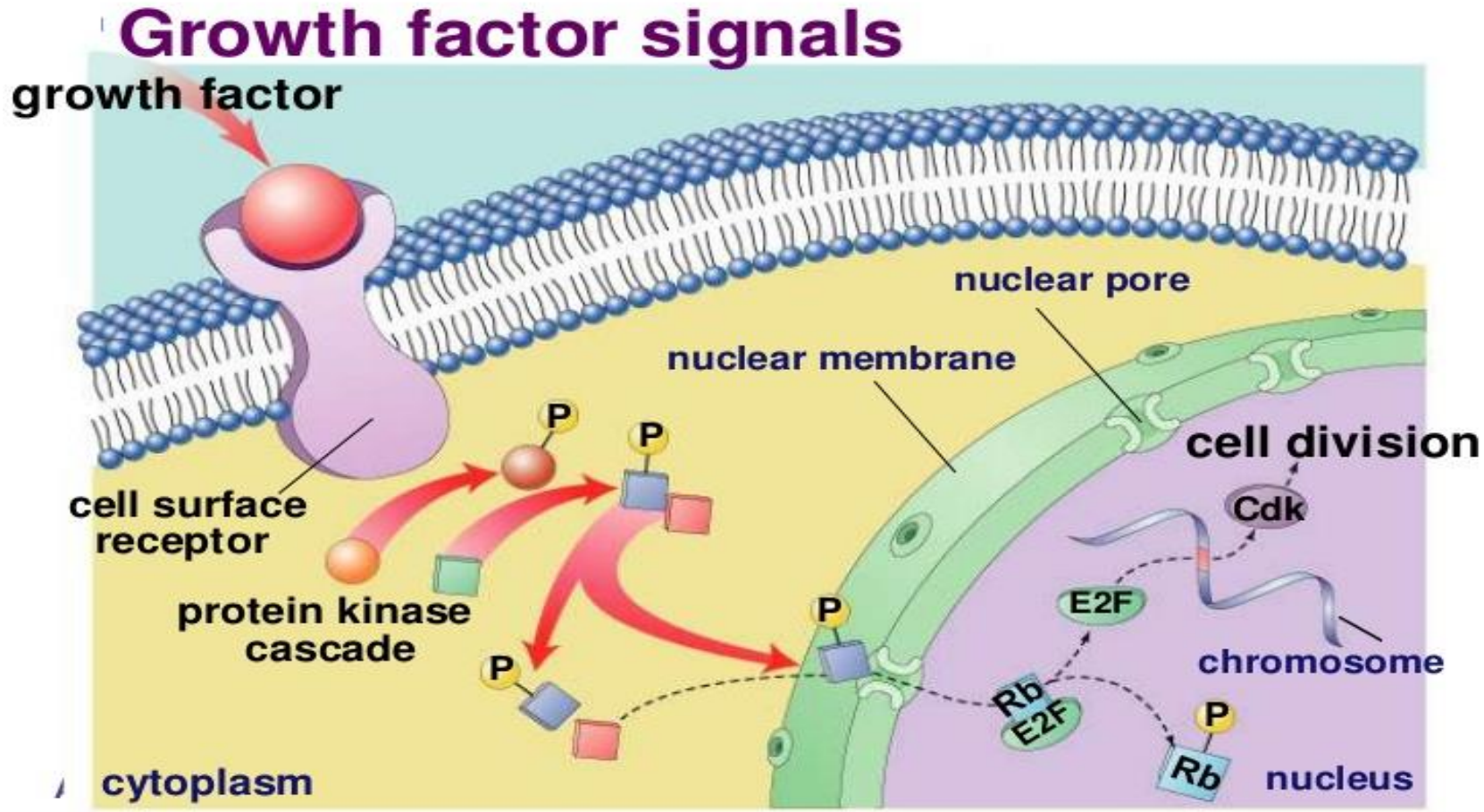
4.1 Growth Factors

- Hematopoietic growth factors are hormone-like substances that stimulate bone marrow to produce blood cells. Shortages of blood cells cause most of the symptoms in people with MDS, the use of growth factors is very appealing.

- Erythropoietin is a growth factor which promotes red blood cells production. Interleukin-11 stimulates platelet production after chemotherapy.

- The sequence of amino acids permits GFs to be placed into families, suggesting that they evolved from a single ancestral protein. The insulin family comprises of somatemedins A and C, insulin, insulin like GF (IGF) and multiplication-stimulating factor (MSF). A 2nd family consists of sarcoma growth factor (SGF), transforming growth factors (TGFs) and epidermal growth factors (EGF).

4.1 Growth Factors



Video illustrating the role of GFs
https://www.youtube.com/watch?v=I3T7Tg_Y90I

Source: Slideshare.net

4.2 Hormones

- **Systemic messengers!**

- Hormones are chemical messengers that carry and travel signals in the blood stream from cells or glands to other tissues and organs so as to maintain chemical levels in the bloodstream that achieve homeostasis. All cellular organisms produce hormones.

- Hormones also regulate the function of their target cells which express a receptor for the hormone. The action of hormones is determined by numerous factors such as its pattern of secretion and the response of the receiving tissue.

- Though few hormones circulate dissolved in the blood-stream, most are carried in the blood, bound to plasma proteins.

- An example of antagonistic pairs of hormones is the Insulin, which causes the level of glucose to drop when it has risen and Glucagon causes blood sugar to rise when it has fallen.

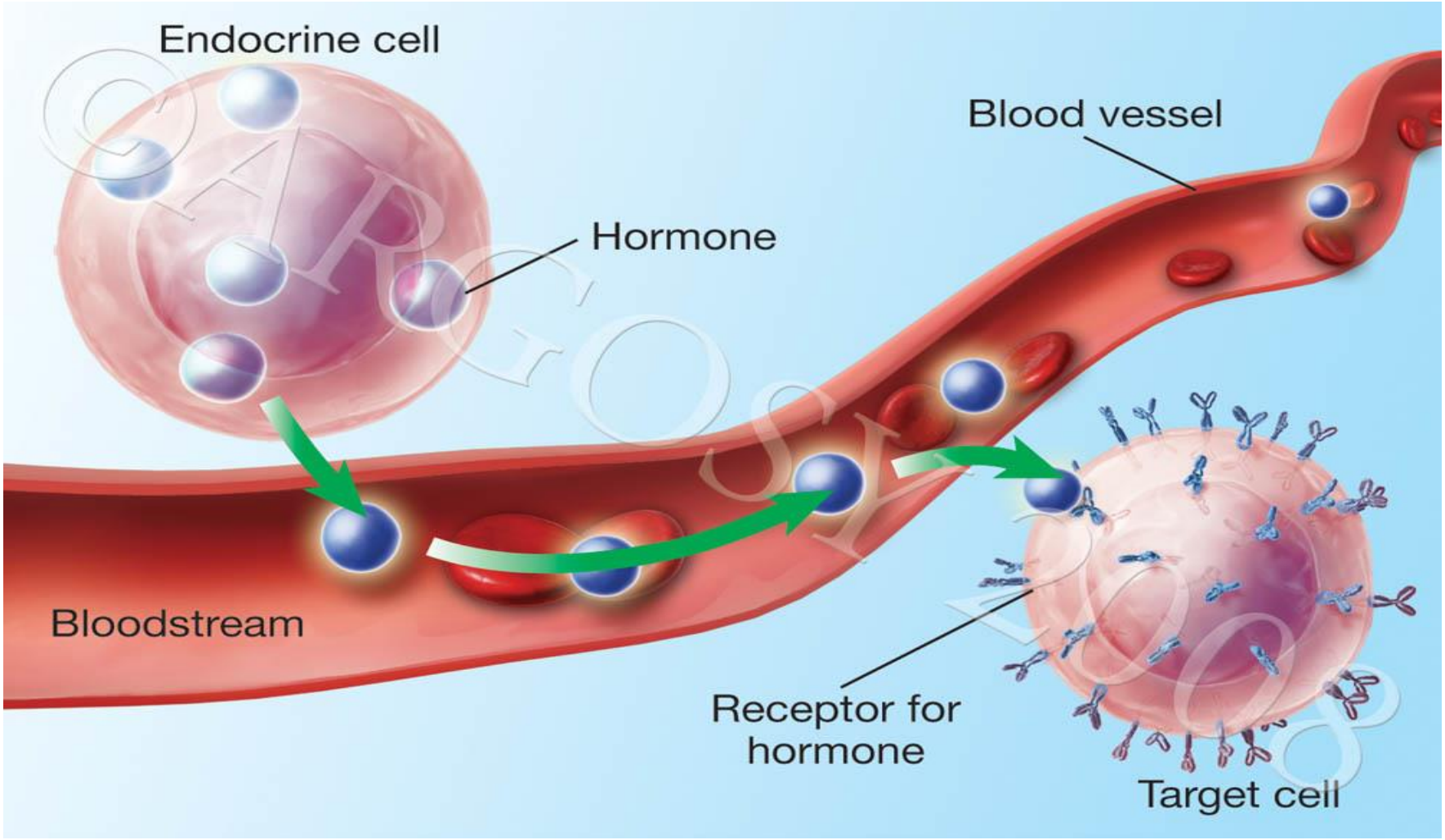
4.2 Hormones

- There are two major classes of hormones.
  - 1. Proteins, peptides and modified amino acids
  - 2. Steroids
- In general, steroids are sex hormones related to sexual maturation and fertility. Steroids are made from cholesterol by placenta by our adrenal gland or gonads.

- Peptides regulate functions such as sleep and sugar concentration. They are made from long strings of amino acids, so sometimes they are referred to as “protein” hormones. Growth hormones for example, help us burn fat and build up muscles.

- Hormones work slowly, over time and affect many different processes in the body such as Growth and development, metabolism, Sexual function and Reproduction mood.

4.2 Hormones



Source:
http://www.argosymedical.com/Cellular/

Mechanisms of Hormones:
https://www.youtube.com/watch?v=TgNwxF3aQpE

4.3 Cytokines

- Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells (Zhang, et al., 2009).

- Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes (Zhang, et al., 2009).

- Cytokines are made by many cell populations, but the predominant producers are helper T cells (Th) and macrophages. Cytokines may be produced in and by peripheral nerve tissue during physiological and pathological processes by resident and recruited macrophages, mast cells and endothelial cells (Zhang, et al., 2009).

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4.3 Cytokines

- Proinflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. There is abundant evidence that certain pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are involved in the process of pathological pain (Xie, et al.,2006).

- IL-1β is released primarily by monocytes and macrophages as well as by nonimmune cells, such as fibroblasts and endothelial cells, during cell injury, infection, invasion, and inflammation (De Leo, et al., 1996).

- IL-6 has been shown to play a central role in the neuronal reaction to nerve injury. Suppression of IL-6R by *in vivo* application of anti-IL-6R antibodies led to reduced regenerative effects. IL-6 is also involved in microglial and astrocytic activation as well as in regulation of neuronal neuropeptides expression (De Leo, et al., 1996).

4.3 Cytokines

- The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response. Cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response (Schafers, et al., 2003).

- Their physiologic role in inflammation and pathologic role in systemic inflammatory states are increasingly recognized. Major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13 (Schafers, et al., 2003).

- Leukemia inhibitory factor, interferon-alpha, IL-6, and transforming growth factor (TGF)-β are categorized as either anti-inflammatory or pro-inflammatory cytokines, under various circumstances. Specific cytokine receptors for IL-1, TNF-α, and IL-18 also function as inhibitors for pro-inflammatory cytokines (Schafers, et al., 2003).

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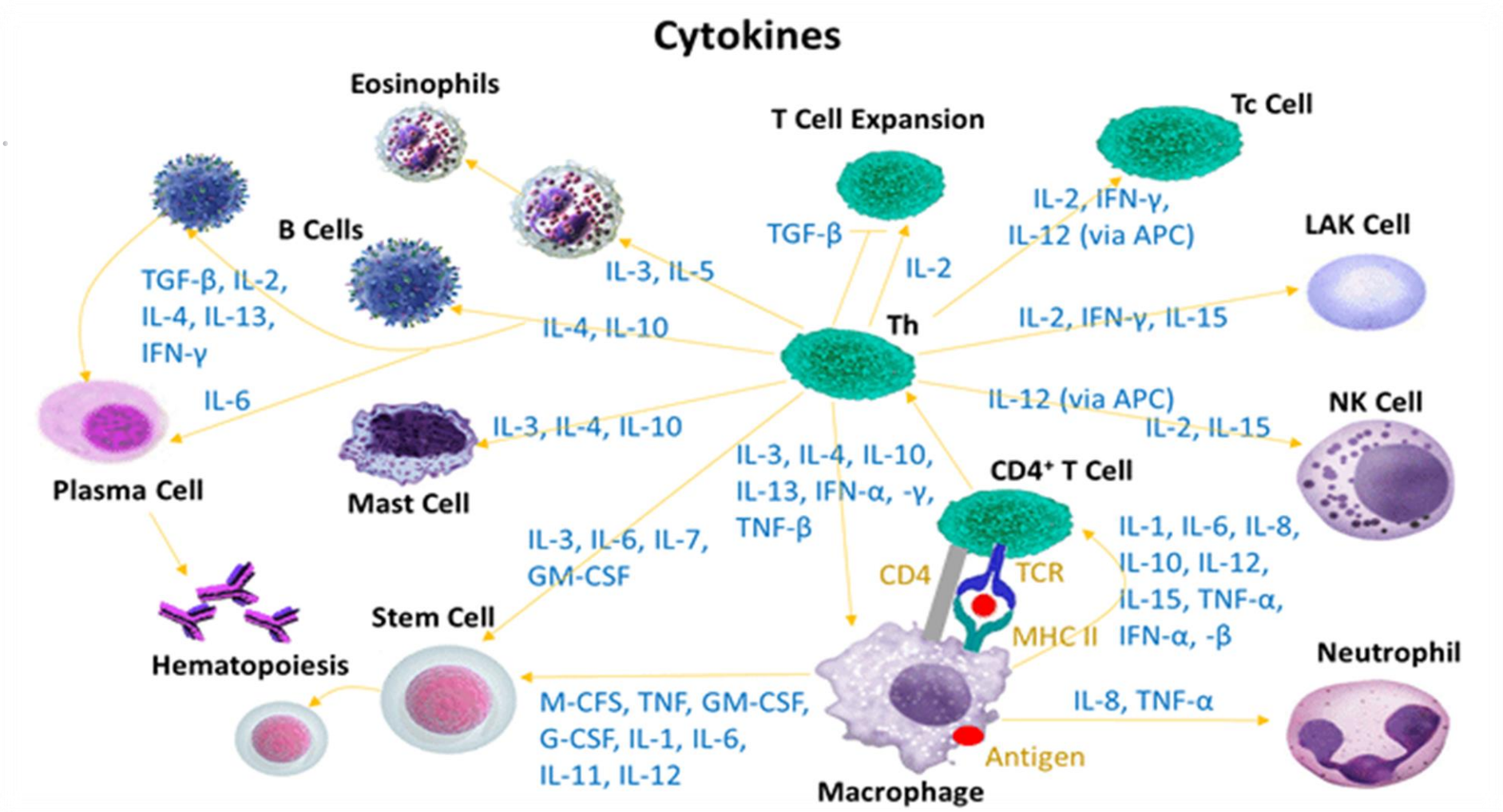
4.3 Cytokines



Source: *https://neobiolab.com*

Video showing a Macrophage Cytokine Release:
https://www.youtube.com/watch?v=KiLJl3NwmpU

Questions?

5. Applications in Tissue engineering

1. Breast reconstruction
2. Cardiovascular system
    1. Cardiac-tissue engineering
    2. Blood vessels
    3. Heart valves
3. Muscoskeletal system
    1. Bone Regeneration
    2. Bone and Cartilage Reconstruction
4. Skin

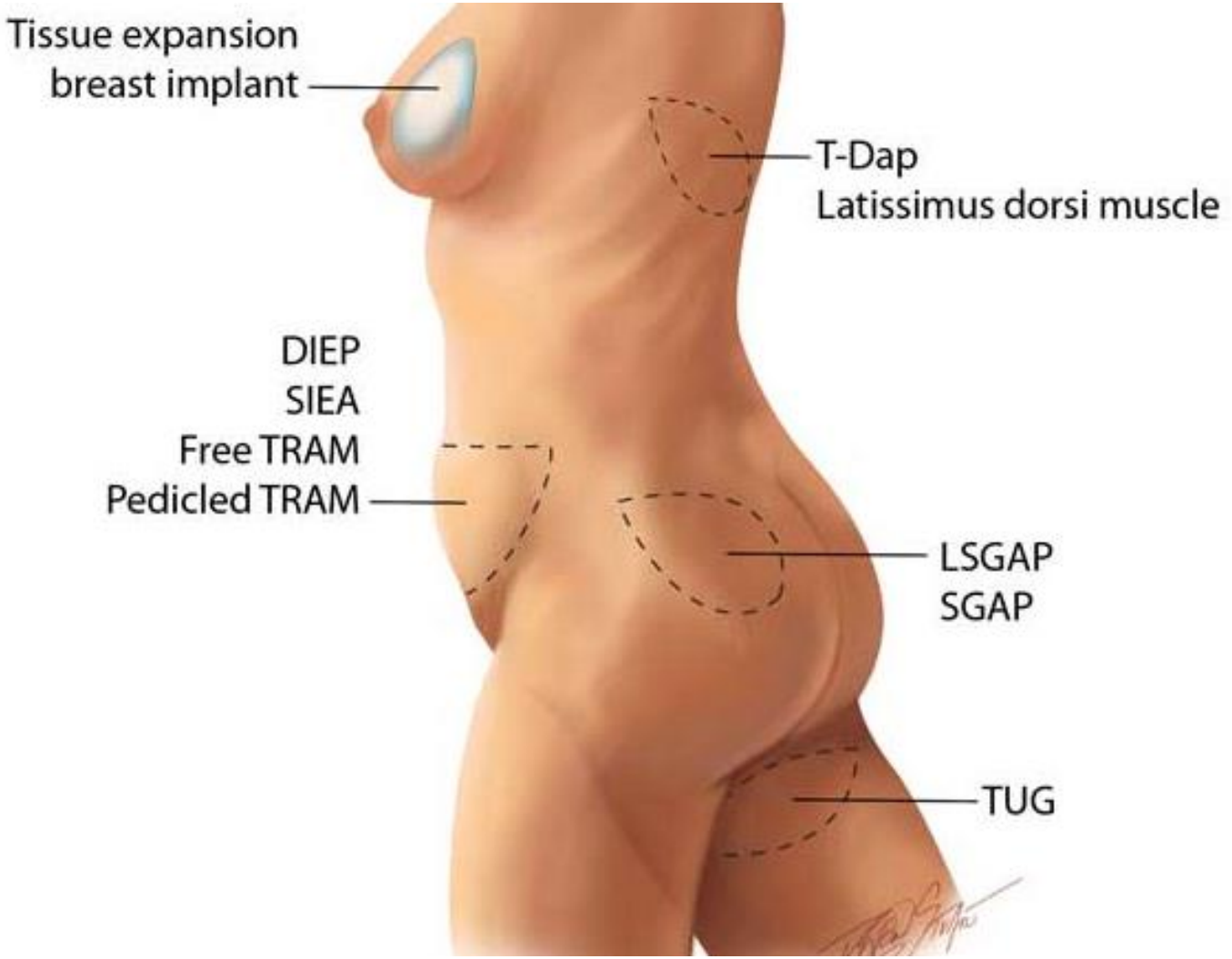
5.1 Breast Reconstruction

Introduction

- Reconstructive surgery after mastectomy or lumpectomy due to breast cancer may benefit greatly from tissue engineering for the creation of new soft-tissue replacements.
- For breast TE, fat tissues (adipocytes) needs to be formed
- Adipocytes are already terminally differentiated and cannot be used for TE (if cell expansion is needed).
- Various cell types can be used for breast reconstruction, including **preadipocytes, fibroblasts, smooth muscle cells, muscle myocytes** and **chondrocytes** (Langer, et al., 2006).
- A cell-polymeric construct can be introduced into the body to provide 3-D support for engineered new tissue. The **implantable forms of materials** are **typically foams, sponges, films** and other **solid devices**. The typical **injectable forms of materials** include **hydrogels and microbeads**. Natural polymers such as **alginate, chitosan, hyaluronic acid** and **collagen** are good examples of injectable materials (Langer., et al., 2006). Two strategies exist:
  - Surgical implanting
  - Deliver of a specific hydrogel material via injection.

- According to ACS, each year, 182,000 new cases of breast cancer are detected that can potentially require breast reconstruction (ACS,2006).

5.1 Breast Reconstruction



Johns Hopkins Medicine

5.1 Breast Reconstruction

- Engineered breast reconstruction can be approached through one of **two basic pathways**:
  1. *In-situ* adipogenesis
  2. *De-novo* adipogenesis

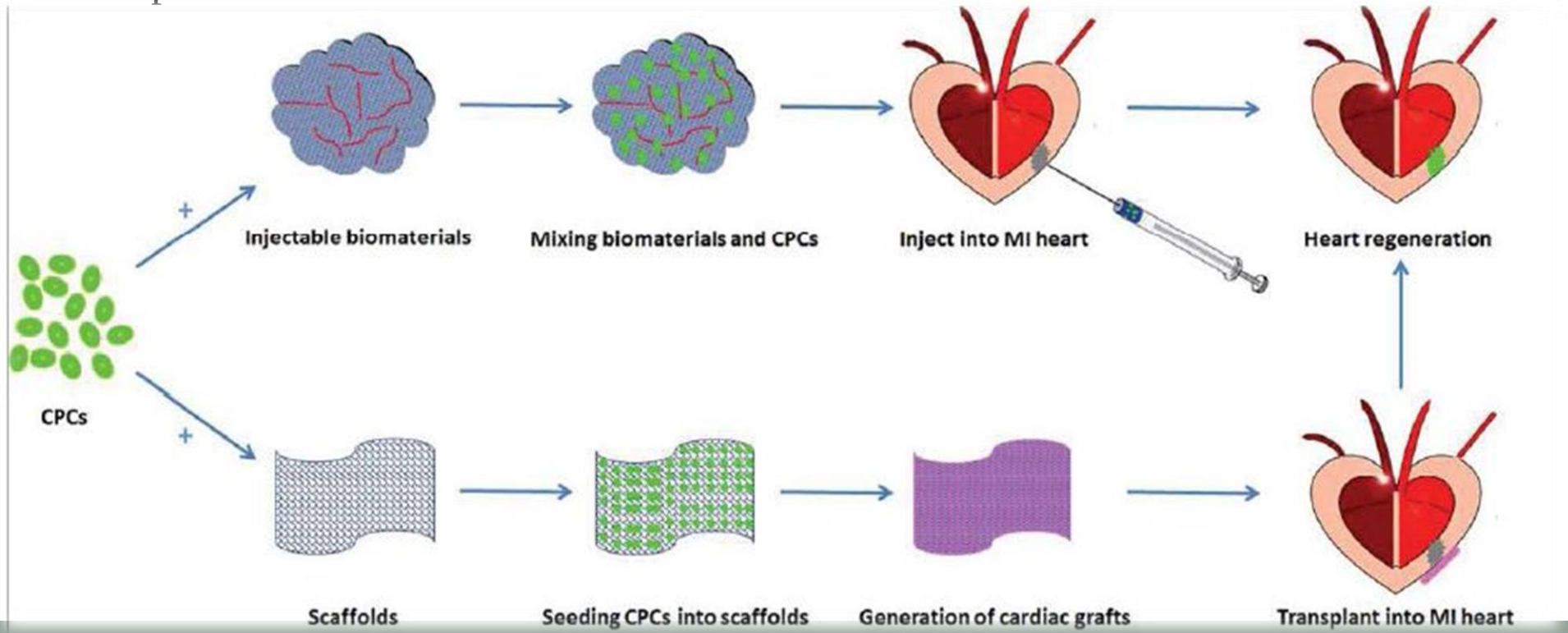
- The first technique employs the transplantation of a supportive matrix to encourage the development of adipose tissue *in-situ*. The matrix is supplemented with specific growth factors that attract native cells, such as fibroblasts and preadipocytes, to migrate into the matrix (Patel and Patrick, 2004).

- In the second approach, tissue-specific cells are isolated from a small tissue biopsy and expanded in-vitro. The cells are subsequently placed onto polymeric scaffolds that act as synthetic ECM. These scaffolds deliver the cells to the desired site in the body, define a space for tissue formation, and potentially control the structure and the function of the engineered tissue (Kim and Mooney, 1998; Mooney, et al., 1996).

5.2.1 Cardiac-Tissue Engineering

Introduction

- Heart disease remains the leading cause of death in developed countries.
- Along with stroke, they are the principal components of cardiovascular disease accounting for nearly 40% of all deaths (Gillum, 1994)
- Tissue engineering offers a potential to grow *in-vitro* functional equivalents of native myocardium for use in tissue repair and to investigate new ways to treat or prevent the disease.



Source: *Tian et al., 2015*

5.2.1 Cardiac-Tissue Engineering

Problem Definition-The myocardium

- The myocardium is a highly differentiated tissue, 1.3-1.6 cm thick in humans, composed of cardiac myocytes and fibroblasts with a dense supporting vasculature and collagen-based extracellular matrix (ECM).

- Cardiac myocytes form a 3-D syncytium that enables propagation of electrical signals across specialized intracellular junctions to produce coordinated mechanical contractions that pump blood forward (Severs, 2000).

- Only 20-40% of the cells in the heart are cardiac myocytes, but they occupy 80-90% of the heart volume. The average cell density in the native rat myocardium in on the order of 5*10^8 cells/cm^3 (Severs, 2010).

M**yocardium - Definition, Function & Anatomy - Human Anatomy:** https://www.youtube.com/watch?v=0bQCPdj_a0o

5.2.1 Cardiac-Tissue Engineering

Problem Definition-Requirements for a functional cardiac patch

- In order to provide a functional cardiac patch, tissue engineering approaches must accurately mimic the structure of native myocardium over several different length scales.

- At the centimeter scale, TE should yield a mechanically stable construct of clinically relevant thickness, comparable to the thickness of the human myocardium (~1.5cm) (Yamanda et al., 1985).

5.2.1 Cardiac-Tissue Engineering

Problem Definition-Clinically Relevant Cell Sources

- The major limitation in the progress of cardiac-tissue engineering toward clinical applications, via either the cardiac patch or drug testing, is the lack of an appropriate human cell source (Soonpaa and Field, 1998).

- Adult cardiac myocytes are terminally differentiated and have no ability to proliferate, thus, they cannot be utilized as a source of autologous cells for tissue engineering(Soonpaa and Field, 1998).

- Cardiac myocytes can be obtained in potentially unlimited quantities and at high purity from embryonic stem cells (Klug et al., 1996). However, nuclear transfer is required to make them autologous, and the presence of undifferentiated cells may lead to teratomas upon implantation (Laflamme and Murry, 2005).

5.2.1 Cardiac-Tissue Engineering

Cell-Sheet Tissue Engineering

- Shimizu et al. (2002) created sheets of neonatal rat cardiomyocytes by plating isolated cells onto a temperature sensitive ply(N-isopropylacrylamide) surface.

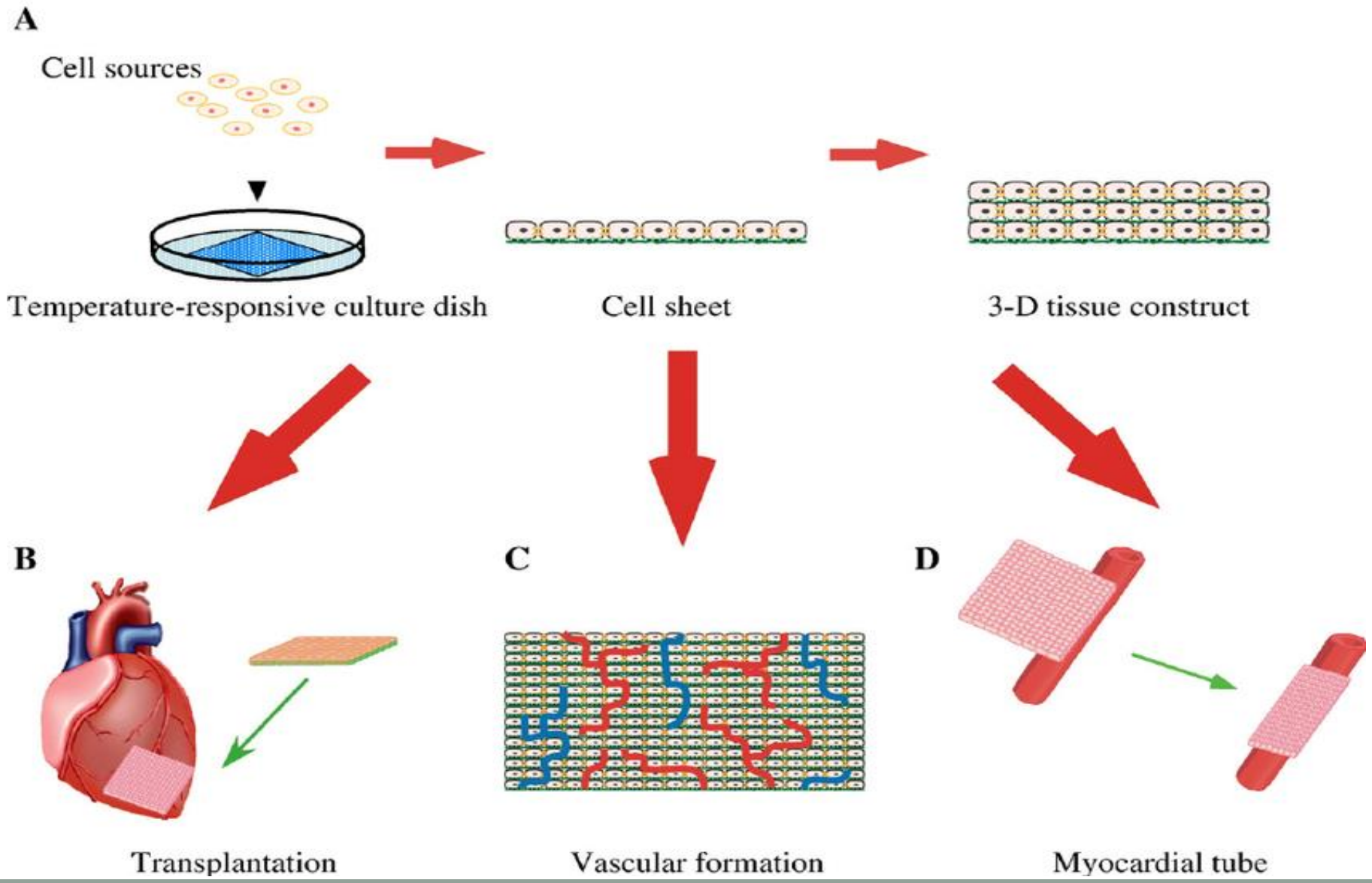
- The change in temperature from 37^{o} C to 32^{o} C causes the cell monolayer to detach . The initial surface area decreases during the detachment approximately fivefold, and the thickness of the cell sheet increases from approximately 20μm to approximately 45μm.

- When the cell sheets were layered over each other, the **electrical communication was established**, as evidenced by electrical signals measured by electrodes and **spontaneous beating** was observed macroscopically.

- When implanted subcutaneously in nude rats, cell sheets survived for up to 12 weeks and vascularized.

5.2.1 Cardiac-Tissue Engineering

Cell-Sheet Tissue Engineering



Source: *Shimuzi et al.,*

5.2.1 Cardiac-Tissue Engineering

Biomimetic approach to Cardiac-Tissue Engineering

- The biomimetic approach involves an integrated use of **cells, biomaterial scaffolds, and bioreactors** to **engineer compact, millimeters thick, synchronously contracting** cardiac-tissue constructs.

- The system design is based on providing:
  1. Convective-diffusive oxygen transport (critical for cell survival and function)
  2. Excitation-contraction coupling (critical for cell differentiation and assembly).

- To mimic the capillary network, culture medium is perfused through a channeled scaffold seeded with cells at a physiologic density

- To mimic the oxygen supply from hemoglobin, the culture medium is supplemented with an oxygen carrier.

- To promote cell differentiation and functional assembly, cultured constructs are subjected to electrical signals designed to mimic those in native heart and to induce synchronous construct contractions (Radisic, et al., 2004b).

5.2.2 Blood Vessels

Introduction

- Principles of tissue engineering are now being applied in the induction and development of microvascular networks as well as capacitance conduits, including the *in-vitro* and *in-vivo* biologic modification of synthetic vascular grafts and the generation of tissue-engineered blood vessels in *bioreactors* and *in-vivo*.

- Alexis Carrel, the father of vascular surgery, was the first to describe the utility and shortfalls of autogenous and synthetic grafts. The main limitation discussed was the lack of durability for small-diameter synthetic grafts.

- Unfortunately, cardiovascular has become more prevalent in the last 100 years, now being the leading cause of death in the world.

- Although autogenous veins or arteries provide the best patency rates for cardiovascular bypass, many patients do not have suitable autogenous vessels available for use.

5.2.2 Blood Vessels

Physicochemical modification on current grafts to improve durability

- The long-term patency of vascular grafts depends on the intrinsic properties of the graft itself and the hemodynamic environment in which the graft is placed as well as patient variables (e.g., diabetes and renal failure) and may or may not be improved by prior or concomitant interventions such as proximal or distal angioplasty.

- On the grounds that tissue incorporation is important for graft function, grafts that have this ability are now desirable for medium and small caliber vessel replacement.

- Polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (ePTFE) are the predominant materials currently used in prosthetic vascular grafts, but both Dacron and ePTFE react with blood components and perigraft tissues in concomitantly beneficial and detrimental fashions.

5.2.2 Blood Vessels

Physicochemical modification on current grafts to improve durability

- All grafts, regardless of their composition and structure, evoke complex but predictable host responses that begin immediately upon restoration of perfusion, and this improved understanding of cellular and molecular components of biomaterial/tissue interactions has led to more intelligent designs of grafts.

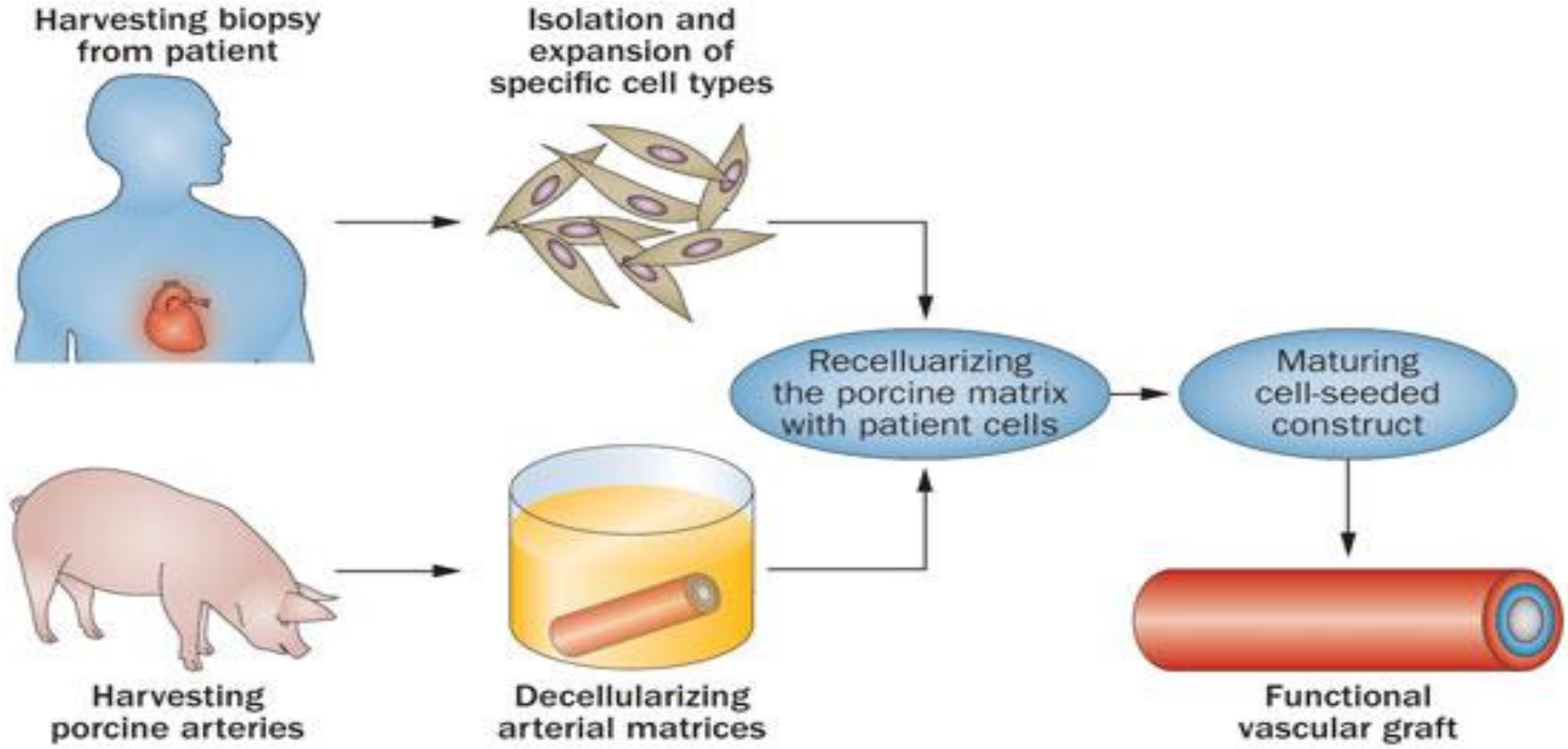
- These approaches include protein adsorptive grafts (growth factors, anticoagulants, antibiotics, etc.) as well as improved graft skeletal construction via synthetic polymers or biologically derived structural proteins that can be bonded to various bioactive cytokines and growth factors to induce a favorable host response (Langer et al., 2006).

Vascular graft for the regeneration of blood vessels:
https://www.youtube.com/watch?v=9bokuAtBJgg

5.2.2 Blood Vessels

Tissue-engineered vascular grafts

- The potential of benefits of tissue engineered vascular grafts (TEVGs) include the creation of a responsive and self-renewing tissue graft with functional intimal, medial, and adventitial layers (including both cellular and ECM components) that can be remodeled by the body according to its needs.



Source: *Seifu et al., 2016*

5.2.2 Blood Vessels

In-vitro Tissue-Engineered Vascular Grafts

- Weinberg and Bell (1986) were the first to develop a TEVG *in vitro*.
- Using collagen and cultured bovine vascular cells, they demonstrated the feasibility of creating a TEVG, but their graft had prohibitively low burst pressures, requiring external Dacron for support.
- In the following decade L'Heureux et al., (1998) constructed a human blood vessel with an acceptable burst strength and a thromboresistant endothelium *in vitro* using cultured umbilical cord-derived human cells.
- However, due to the immunogenic effects of the heterogeneic ECs *in vivo*, their graft, devoid of ECs had only 50% patency rate at 8 weeks in a canine model.
- Since neonatal cells have a greater rejuvenative capacity, the foregoing TEVG was not considered applicable to the aged population that would benefit from a TEVG.

5.2.2 Blood Vessels

In-vitro Tissue-Engineered Vascular Grafts

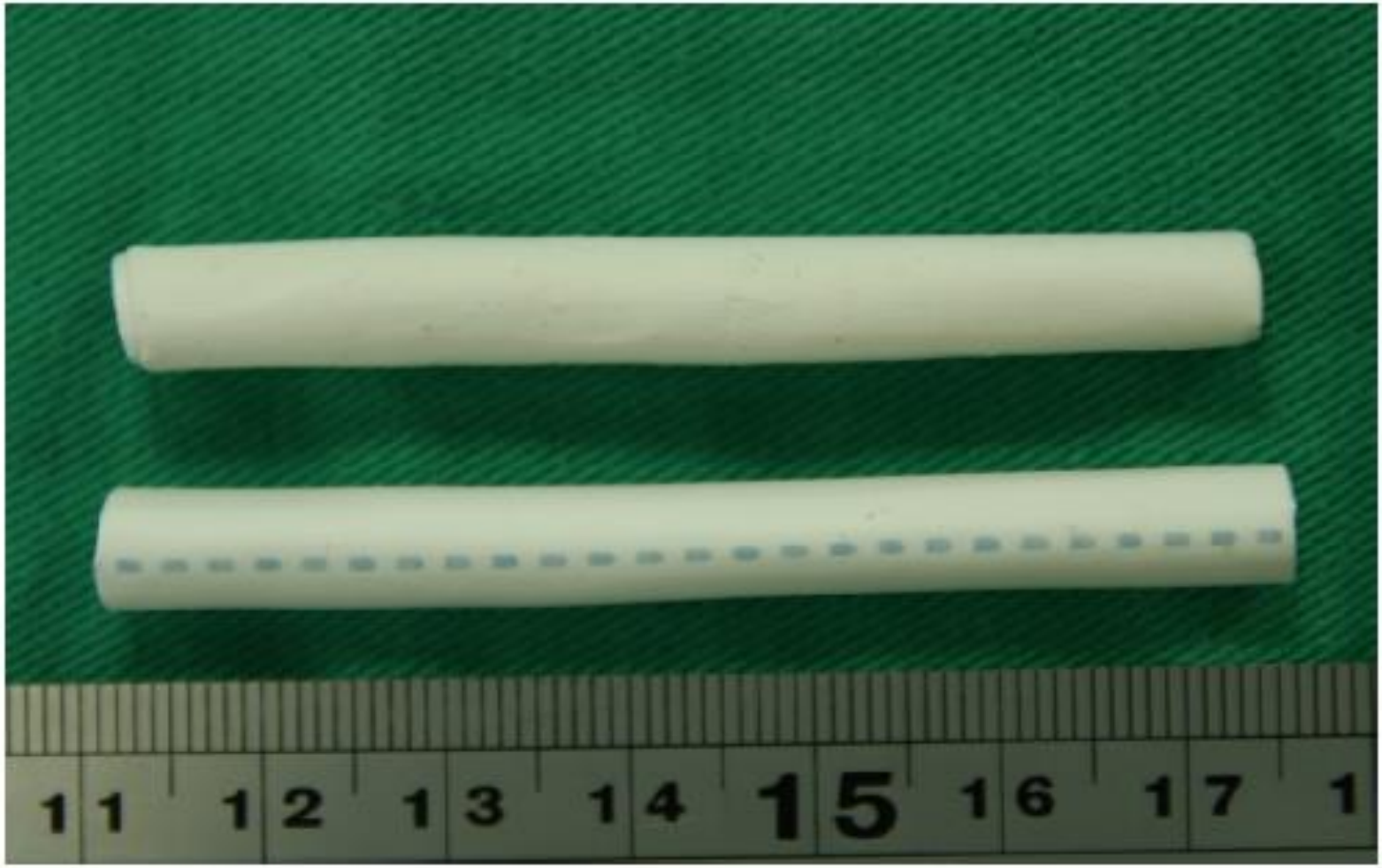


Figure 2 Photograph of the vascular prosthetic grafts (top, loaded with nanofibers; bottom, bare graft).

Source: *Liu, et al.,*

5.2.3 Heart Valves

Introduction

- Heart valve disease is a considerable medical problem around the world.
- The American Heart Association has estimated that 87,000 heart valve replacements procedures were performed in the US in 2000, and nearly 275,000 procedures are performed annually.
- The treatment for end-stage heart valve disease is valve replacement; however, the best current replacement heart valves suffer from significant shortcomings.
- Promising alternatives to current replacement heart valves are being developed in the field of tissue engineering.
- This multidisciplinary effort comprises the areas of **tissue biomechanics, immunology, injury response, cellular and tissue development, chemical, physical and pharmacological manipulation** of both cells and biomaterials.

5.2.3 Heart Valves

Application of tissue engineering toward the construction of a replacement heart valve

- The ideal heart valve replacement would be readily available and perfectly biocompatible, have the potential for growth and be durable (Mayer, 2001).

- The manufacture of an autologous, tissue engineered heart valve could potentially be a considerable improvement over the best current technology.

- It would be capable of utilizing the natural mechanisms for repair, remodeling and regeneration and thereby by highly durable.

- In addition, it would possess the potential for growth. Since the cells could be harvested from the patient in need of a valve, immune-mediated rejection would be eliminated. In short, tissue engineered heart valve could be the ideal replacement heart valve (Shalak and Fox, 1988).

5.2.3 Heart Valves

Tissue Engineering Theory Applied to Heart Valves

- The semilunar heart valve is not completely avascular and nutrients and oxygen are supplied via two complementary pathways. The thickest section of the valve is vascularized for transport of oxygen and nutrients (Weing et al.,2001).

- An advantage of tissue engineering a semilunar heart valve is its composition of easily cultured cells. This is a critical characteristic because it allows for the *in vitro* isolation and *in vitro* expansion of autologous cells.

- Another critical feature enabling the development of a tissue-engineered heart valve is the fact that valve function can be readily studied using well-established techniques, such as Doppler ultrasonography and arteriography (Mayer, 2001)

5.2.3 Heart Valves

Biomaterials and Scaffolds

- Tissue engineering scaffolds must be **biocompatible, biodegradable** with safe by-products and **highly porous** yet sufficiently mechanically stable to appropriate function (Rabkin-Aikawa, et al., 2005).

- The scaffold must possess properties that promote cell attachment and recapitulate complex tissues.

- Scaffolds can be made from either synthetic or natural materials. Natural biomaterials are usually composed of ECM components:
  1. **Collagen**
  2. **Fibrin**
  3. **Elastin**
  4. **Glycosaminoglycans**
  5. **Decellularized heart valve**

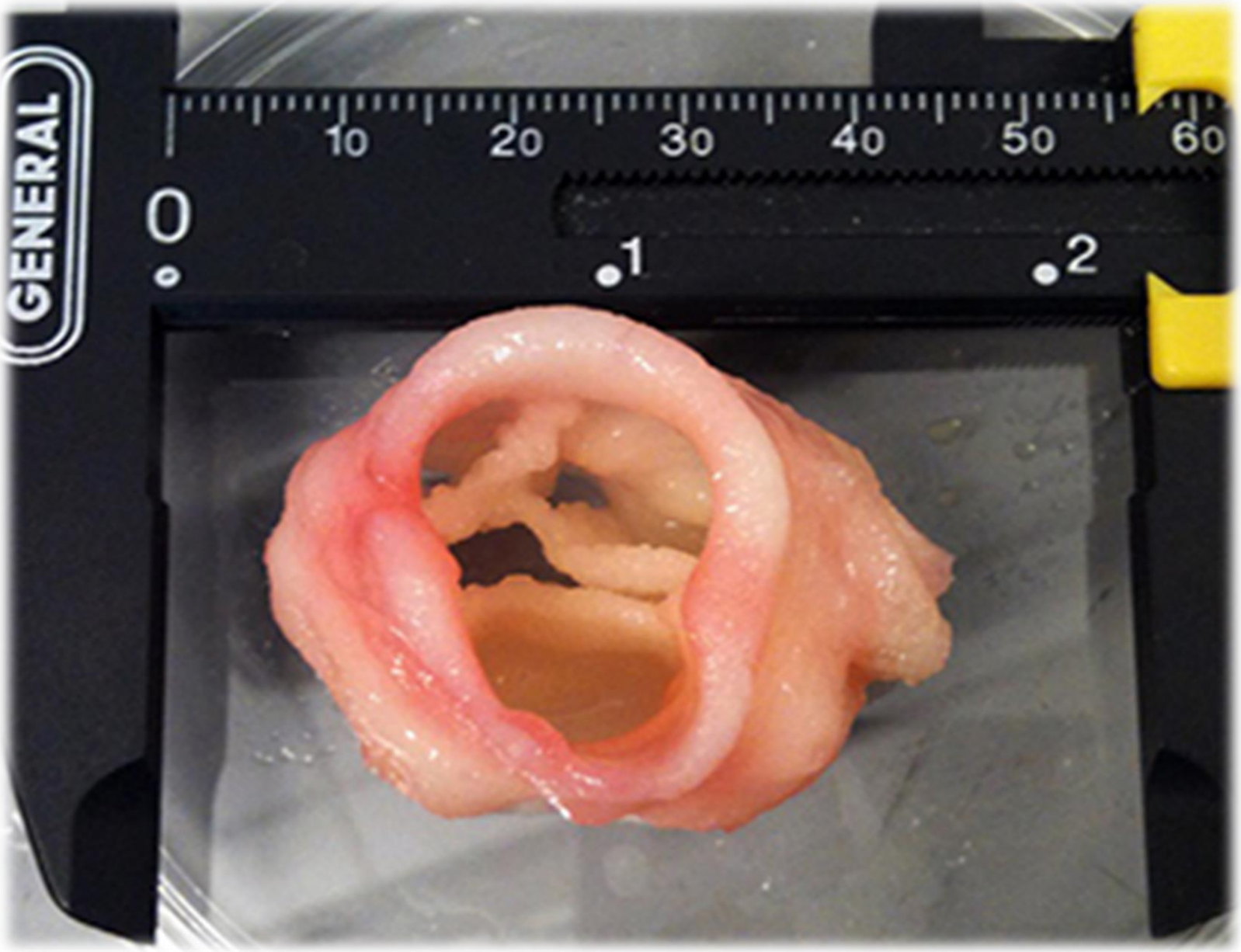
5.2.3 Heart Valves

Biomaterials and Scaffolds

- The most common synthetic polymers in tissue engineering include the poly α-hydroxyesters poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA) and copolymers poly(Lactic-co-glycolic acid) (PLGA), poly(anhydrides) and poly(peptides).

- Another popular polymer is PHA, or polyhyroxyalkanoate (Rabkin-Aikawa et al., 2005). PHA is a thermoplastic that is biocompatible, resorbable and flexible and that causes minimal inflammatory response (Williams et al., 1999).

5.2.3 Heart Valves



Creating Valve Tissue Using 3-D Bioprinting ***Source:*** *Butcher et al., 2012*

5.2.3 Heart Valves

Neotissue Development in the Tissue-engineered Heart Valve

- Upon the formation of the cell-scaffold construct, neotissue begins its development. The process, however, is poorly understood and is controlled by many factors (Shalak and Fox, 1988).

- The environment of the growing construct will influence the ECM and histological structure formed.

- Scientists have approached this phenomenon from two perspectives.

- The first is a “blackbox” approach, where the scaffold is used as a cell delivery device and implanted *in vivo* shortly after cell attachment has taken place (Langer and Vacanti., 1993).

- In a second approach, an instrument called bioreactor is used.

5.2.3 Heart Valves

Neotissue Development in the Tissue-engineered Heart Valve

- One type of bioreactor used in tissue engineering a heart valve is the pulse duplicator. This pulsatile bioreactor supplies physiological flow and pressure to the developing tissue-engineered heart valve and promotes both the modulation of cellular function and the development of mechanical strength (Nicklason., et al., 1999).

- Neotissue made in this way possesses superior mechanical properties over unstrained controls and results in heart valve cusps that are considerably less stiff than static controls (Gloeckner et al., 1999).

5.2.3 Heart Valves

Conclusion

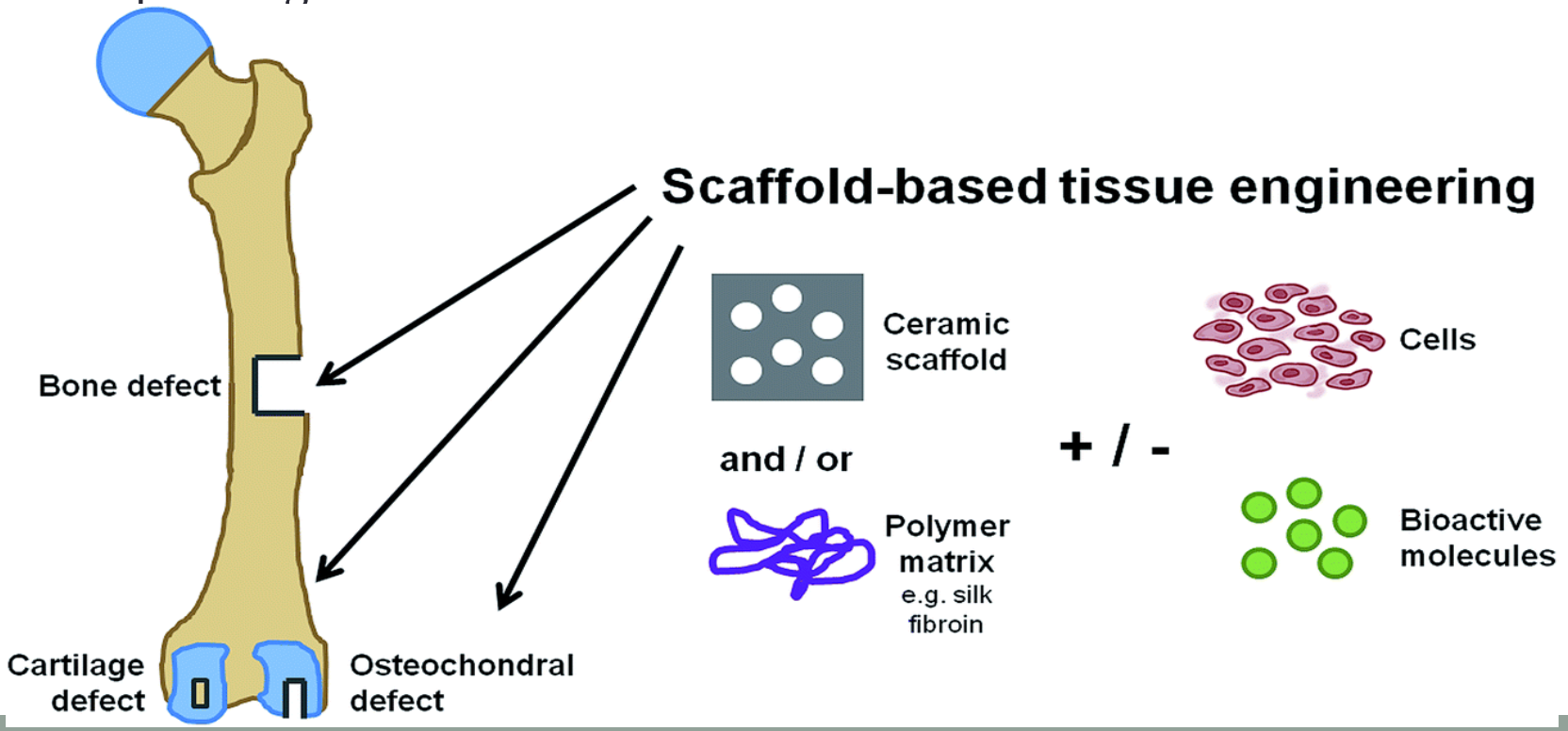
- Successful development of a tissue-engineered replacement heart valve may hold the key to better treatment of end-stage valve disease.
- Although significant progress has been achieved since its inception in the early 90s, the field is young and many key issues need to be resolved.
- As for yet, we are still limited by our knowledge of cell biology and ECM production and maintenance of a normal valve.
- The identification of an ideal cell source and optimal matrix persists as the point of focus in tissue-engineering strategy.
- It is possible that a more complete understanding of embryonic and fetal heart valve development may provide insight that eventually enables tissue engineers to build consistently clinically acceptable replacement heart valves *ex* vivo (Flanagan and Pandit, 2003).

5.3.1 Bone Regeneration

Introduction

- Research in bone regeneration truly took off in the 1960s when Burnwell at al., confirmed bone marrow's osteogenicity and Urist discovered bone morphogens.

- Scientific advances in bone-tissue engineering was thus achieved through both in-depth understanding of bone cell biology and commercial-scale generation of bone specific growth factors.



Source: *Jiao Li, et al.,*

5.3.1 Bone Regeneration

Cell-Based Therapy

- The clinical use of cell-based therapies to aid in bone regeneration utilized the use of bone marrow injections as a source of stem cells into nonunions (Phemister, 1947).

- In the 1960s, Burwell (1964) further defined the bone regeneration potential of the iliac crest autograft as a function of the crest's marrow content. Since that time, while plain bone marrow injections are still in some cases the therapeutic option du jour, scientific developments provided health care professionals with(Langer et al., 2006):

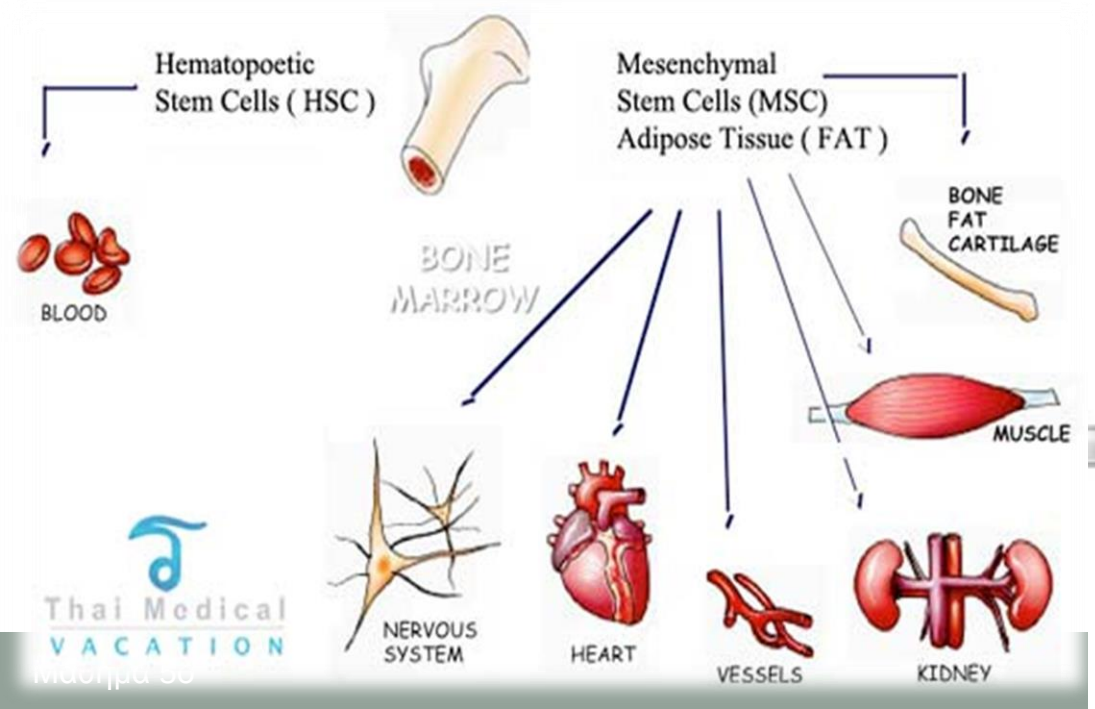
1. Optimized matrices to attach and maintain bone marrow cells
2. Point-of-care bone marrow cell concentration methods
3. Allogeneic, culture-expanded stem cell concentrates

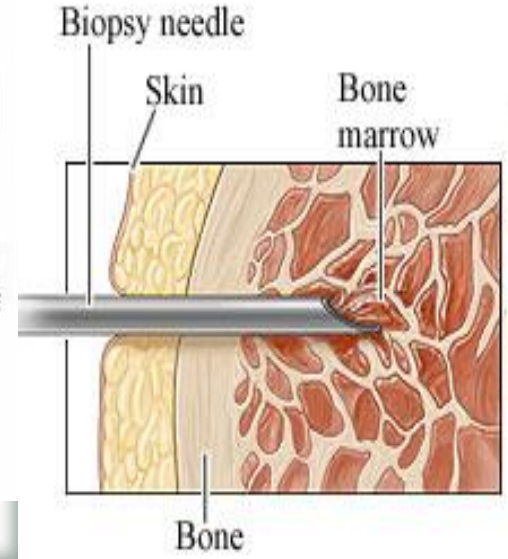
5.3.1 Bone Regeneration

Cell-Based Therapy-Use of Bone Marrow as a Source of Stem Cells

- Of all human tissues, bone marrow was found to be particularly advantageous from a cell-sourcing perspective, for it can be obtained from the iliac crest using a noninvasive procedure with a simple needle aspiration (Langer, et al., 2006).

- More importantly, it is self-renewing and was found to regenerate its own nucleated cellular content within 90 days (Stroncek, et al., 1991).





5.3.1 Bone and Cartilage Reconstruction

Introduction

- Tissue engineering offers a better approach than conventional surgery for bone and cartilage repair by reducing donor site morbidity (Langer et al., 2006).

- To achieve this goal, animal study of repairing clinically relevant tissue defects, especially in large animals, has become a crucial step.

- One of the disadvantages of traditional surgical repair procedure is the need to harvest autologous tissues from a donor site of the human body for the repair of a defect on another site, thus leaving a secondary tissue defect (Langer et al., 2006).

- Tissue engineering offers an approach for tissue repair and regeneration without the necessity of donor site morbidity (Langer et al., 2006).

5.3.1 Bone and Cartilage Reconstruction

Bone Reconstruction

- As early as 1993 Vacanti et al. applied tissue engineering techniques to the construction of bone grafts in the subcutaneous tissue of nude mice using periosteum derived osteoblasts and degradable polymer scaffold, which clearly proved the concept that the tissue engineering approach can regenerate bone tissue in-vivo.

- Kaplan's group used silk as a scaffold material for bone engineering (Meinel et al., 2005).

- In their study, hMSC were isolated and seeded on a porous silk fibroin scaffold, *in-vitro* engineered for 5 weeks in a bioreactor and then transplanted to repair the calvarial bone defects created in nude mice with a diameter of 4 mm.

- Their results showed that hMSC could undergo osteogenic differentiation on this novel scaffold and that *in-vitro* engineered bone could achieve better reparative results than the cell-scaffold complex without *in-vitro* engineering.

5.3.1 Bone and Cartilage Reconstruction

Cartilage Reconstruction

- In 1997, Cao *et al*. reported that cartilage with a complicated 3D structure, such as the human ear shape, could be engineered using nude mice as an animal model, which reveals the great potential for the clinical application of engineered cartilage (Cao et al., 1997).

Video showing the mice with the "extra ear" : https://www.youtube.com/watch?v=PEc7QXAjsL4

Source: *Cao et al., 1997*

5.3.1 Bone and Cartilage Reconstruction

Cartilage Reconstruction

- With regard to clinically relevant models, for cartilage engineering and repair, articular cartilage repair is the most commonly involved subject.
- Liu et al. had as a center the repair of the osteochondral defect in large mammals using autologous chondrocyte-engineered cartilage (Liu et al., 2002).
- In a porcine mode, autologous articular cartilage was harvested from the knee joint on one side. On the other side, an 8-mm full-thickness articular cartilage defect deep to the underlying cancellous bone was created.
- The cell-scaffold construct containing PGA, polyethylene-polypropylene hydrogel, and chondrocytes was then transplanted to repair the defects in the experimental group.
- Grossly, cartilage tissues were formed in the defects of the experimental group as early as 4 weeks after transplantation.
- At 24 weeks postrepair, gross examination revealed a complete repair of the defects by engineering cartilage, shown by a smooth articular surface indistinguishable from nearby normal cartilage (Liu et al., 2002).

5.5 Skin-Wound Repair

Introduction

- The skin is the largest organ in the body, and its primary function is to serve as a protective barrier against the environment (Langer et al., 2006).

- Other important functions of the skin include fluid homeostasis, thermoregulation, immune surveillance, sensory detection and self-healing. Loss of integrity of large potions of the skin due to injury or illness may result in significant disability or even death (Langer et al., 2006).

- It is estimated that in 1992, there were 35.2 million cases of significant skin loss (Wound Care, 1993) that required major therapeutic intervention.

- Of these, 7 million wounds became chronic.

5.5 Skin-Wound Repair

Introduction

- The most common single cause of significant skin loss is thermal injury, which accounts for an estimated 1 million emergency room visits per year (American Burn Association, 2005).

- Other causes of skin loss include trauma and chronic ulcerations secondary to diabetes mellitus, pressure and venous stasis (Langer et al., 2006).

- In 2003, a survey estimated the U.S market for advanced wound care products, including biological and synthetic dressings, to be greater than 1.7 billion dollars.

5.5 Skin-Wound Repair

Tissue Engineered Therapy: Established practice

- Initial attempts to speed up wound repair and improve the quality of healing in chronic or burn wounds involved the use of synthetic, composite synthetic or biological dressings (Broomberg et al., 1965; Purna and Babu, 2000).

- Although effective, these dressings did not offer any permanent treatment, since eventually an autograft had to be implanted to achieve complete healing, which is often undesirable due to donor site morbidity.

- The advent of TE constructs has, however, revolutionized the wound-healing practice (Rosso et al., 2005; Simpson, 2006). These constructs could be classified into two main categories: **cellular** and **acellular** (Langer et al., 2006).

- Regardless of whether they are cellular or acellular, the basic block of theses constructs is composed of a biomimetic and a scaffolding material.

5.5 Skin-Wound Repair

Tissue Engineered Therapy: Established practice

- While the biomimetic functions to stimulate cells to perform their physiological functions, the scaffold typically provides a mechanical support for the cells to spread on and to proliferate within, to produce new tissue (Langer et al., 2006).

- However, scaffolds prepared from naturally occurring biopolymers may provide additional biological stimuli to support cell and tissue function (Lutolf and Hubbell, 2005).

- After implantation, these constructs promote faster healing, resulting in the development of a new tissue that bears a close structural and functional resemblance to the uninjured, host tissue

5.5 Skin-Wound Repair

Tissue Engineered Therapy: New Approaches

- A general perception of novel engineering approaches emerges from suggestions such as development of **easy-to-handle, user-friendly, cost effective, acellular** that are based on nonanimal products.

- Since tissue cells are the primary source of various ECM molecules that facilitate and synchronize tissue repair, any acellular product must, therefore, be conductive to recruiting the host tissue cells rapidly and inductive to stimulating the invading cells to proliferate, synthesize new ECM, and, if required, differentiate (Langer et al., 2006).

- Hyaluronan is a nonsulfated GAG present in most human tissues. During wound repair, it serves multiple important functions, ranging from regulating inflammation to promoting fibroblast migration and proliferation (Chen and Abatangelo, 1999).

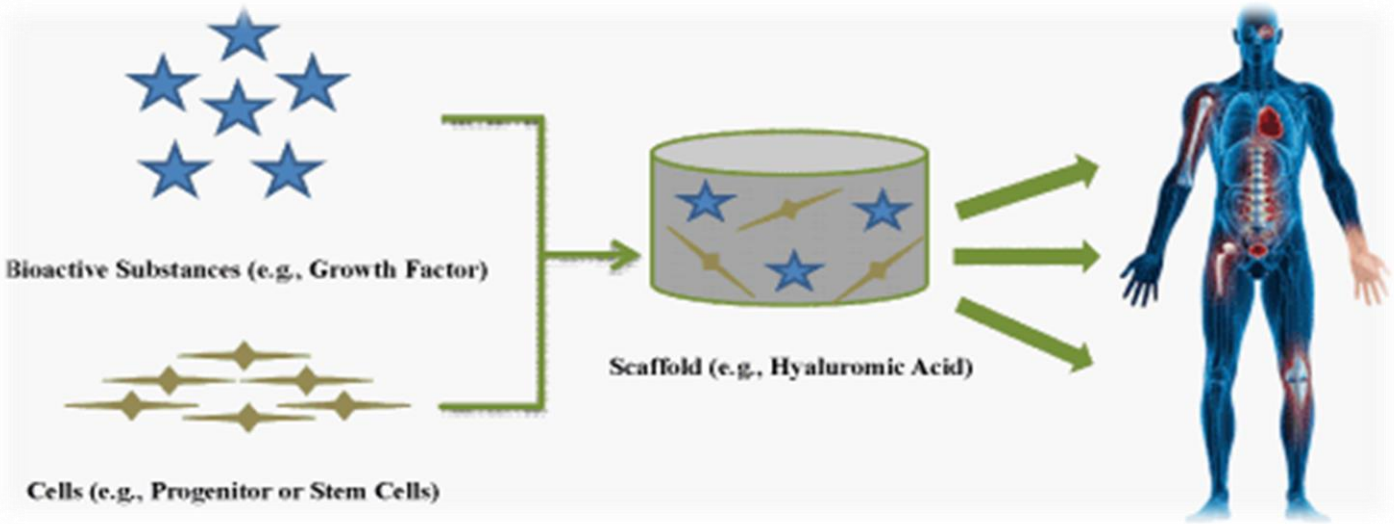
5.5 Skin-Wound Repair

Tissue Engineered Therapy: New Approaches

- Similar to synthetic polymers, hyaluronan can be chemically modified to obtain a variety of stable derivatives (Prestwich, et al., 1998).

- Therefore, by offering the advantages of both natural and synthetic materials, hyaluronan promises to be a suitable scaffolding material for acellular matrices.

- Indeed, chemically modified hyaluronan scaffolds have been successfully used for various tissue-engineering applications, including wound healing (Campoccia et al., 1998; Kirker et al., 2002).



Source: *Kirker et al., 2002*

5.5 Skin-Wound Repair

Tissue Engineered Therapy: New Approaches

Engineering a second skin:
https://www.youtube.com/watch?v=AkpT5BihMio

Source: *Yannas MIT*

Questions?